

Cognitive function, the brain and glucocorticoids

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To Karen and Maeve

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Declaration

The research described in this thesis was the unaided work of the author, except where acknowledgement is made by reference. No part of this work has previously been accepted for any other degree, nor is any part of it being concurrently submitted in candidature for another degree.



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Abstract

Several domains of cognitive function show lower mean scores and increasing variability with increasing age. Little is known about the biological mechanisms underlying these changes. There is evidence, largely from animal studies, that prolonged exposure to high levels of glucocorticoids is associated with (a) atrophy of brain regions known to be essential for cognitive functioning, such as the hippocampus, and (b) decrements in cognitive function with ageing. There are few human studies examining these links. The studies in this thesis were aimed at testing the hypotheses that elevated levels in cortisol are associated with relative cognitive impairment, with relative atrophy of the hippocampus and other brain regions, and also with variations in the levels of brain metabolites, and also that cognitive function is associated with brain size and metabolites. Additionally, measures of glucose homeostasis (fasting glucose and glycosylated haemoglobin (HbA1c)) were hypothesised to be negatively correlated with cognitive function. 111 healthy, unmedicated men aged 65-70 were recruited. Subjects had blood taken for 9am, 2.30pm, and post-dexamethasone (0.25mg) cortisol levels, fasting glucose, and HbA1c, and did a battery of cognitive tests, including tests of 'premorbid' intelligence, fluid intelligence, verbal and visuospatial memory and processing speed. 100 of the subjects underwent two modalities of neuroimaging: (a) structural magnetic resonance imaging, with intracranial area, hippocampus, temporal lobe and frontal lobe volumes measured, and (b) magnetic resonance spectroscopy (MRS), with N-acetylaspartate (NAA), choline (Cho) and creatine (Cr) levels measured. Principal components analyses showed that a single component (designated the 'general cognitive factor') accounted for 51% of the variance in cognitive performance; rotation yielded two correlated components representing fluid intelligence / visuospatial memory tasks, and verbal memory tasks. Intracranial area and several regional brain volumes correlated positively and significantly with 'premorbid' and fluid intelligence and visuospatial memory. Verbal memory and verbal fluency did not correlate significantly with any brain volumes. Structural equation modelling showed that the relationships between cognitive tests and brain

volumes could best be summarised by a significant positive relationship between overall brain size and the general cognitive factor ($r=0.42$, $p<0.05$), and not by associations between individual tests and particular brain regions. Both NAA/Cr and Cho/Cr ratios correlated positively with tests of verbal memory and a verbal memory factor (e.g. NAA/Cr and Logical Memory: $r=0.24$, $p<0.05$). Cho/Cr ratios also correlated positively with visuospatial memory (eg. Visual Reproduction: $r=0.21$, $p<0.05$). There were several small but statistically significant correlations in the predicted (negative) direction between brain volumes and cortisol levels. Left temporal lobe volumes correlated with 9am cortisol ($r=-0.22$) and 2.30pm cortisol ($r=-0.26$), right temporal lobe volumes correlated with 9am cortisol ($r=-0.21$), right hippocampal volumes correlated with 9am cortisol ($r=-0.22$) and post-dexamethasone cortisol ($r=-0.24$). These correlations were significant at $p<0.05$, 2-tailed. There were no significant correlations between cortisol measures and metabolite ratios (from MRS). Correlations between cortisol measures and cognitive tests were largely in the predicted direction, though few of these correlations reached conventional levels of statistical significance. The general cognitive factor and the fluid / intelligence factor, adjusted for 'premorbid' intelligence, correlated significantly and negatively with 9am cortisol levels, at $r=-0.23$ ($p=0.028$, 2-tailed). HbA1c was significantly negatively correlated with two measures of verbal memory, but not with other cognitive tests (list-learning: $r=-0.24$, $p=0.01$; delayed paragraph recall $r=-0.31$, $p=0.018$, 2-tailed). These results demonstrate that in healthy, elderly men, overall brain size and metabolite ratios are significantly related to cognitive ability. A possible mechanistic link between these two domains is variations in cortisol with ageing. The results of the present studies are supportive of the hypothesis that elevated glucocorticoids are associated with ageing-related changes in brain volumes, and, less clearly, cognitive function. Follow-up studies will help determine whether cortisol levels are predictive of worsening brain atrophy and cognitive decline.

Publications arising from this thesis

MacLullich AMJ, Ferguson KJ, Deary IJ, Seckl JR, Starr JM, Wardlaw JM (2002) Intracranial capacity and brain volumes are associated with cognition in healthy elderly men. *Neurology* 59: 169-174

Marshall I, Bruce SD, Higinbotham J, MacLullich A, Wardlaw JM, Ferguson KJ, Seckl J (2000) Choice of spectroscopic lineshape model affects metabolite peak areas and area ratios. *Magnetic Resonance in Medicine* 44: 646-649

Ferguson KJ, MacLullich AM, Marshall I, Deary IJ, Starr JM, Seckl JR, Wardlaw JM (2002) Magnetic resonance spectroscopy and cognitive function in healthy elderly men. *Brain* 125: 2743-2749

MacLullich AMJ, Deary IJ, Starr JM, Walker BR, Seckl JR (2003) Glycosylated hemoglobin levels in healthy elderly non-diabetic men are negatively associated with verbal memory. *Journal of the American Geriatrics Society* (in press)

Abbreviations

11 β -HSD1: 11 β -hydroxysteroid dehydrogenase type 1

11 β -HSD2: 11 β -hydroxysteroid dehydrogenase type 2

ACTH: adrenocorticotrophic hormone

AD: Alzheimer's disease

AVLT: Rey Auditory-Verbal Learning Test

BVRT: Benton Visual Retention Test

Cho: choline

Cr: creatine

CRH: corticotrophin-releasing hormone

DM: diabetes mellitus

DSST: Digit-Symbol Substitution Test

g: general intelligence

Gc: crystallised intelligence

Gf: fluid intelligence

GR: glucocorticoid receptor

H1-MRS: proton magnetic resonance spectroscopy

HbA1c: glycosylated haemoglobin

HPA: hypothalamo-pituitary-adrenal

ICA: intracranial area

IQ: intelligence quotient

MMSE: mini-mental state examination

MR: mineralocorticoid receptor

MRI: magnetic resonance imaging

NAA: N-acetylaspartate

NART: National Adult Reading Test

P31-MRS: phosphorus-31 magnetic resonance imaging

PCA: principal components analysis

PCr: phosphocreatine

RSPM: Raven's Standard Progressive Matrices

WAIS-R: Wechsler Adult Intelligence Scale-Revised

Materials

Cognitive test materials

These were obtained from the standard commercial or public-domain sources:

Benton Visual Retention Test: Psychological Corporation, New York

National Adult Reading Test: NFER-Nelson, London

Raven's Standard Progressive Matrices: HK Lewis & Co., London

Verbal Fluency: public domain (Lezak, 1995)

Wechsler Adult Intelligence Scale – Revised: Psychological Corporation, New York

Wechsler Memory Scale – Revised: Psychological Corporation, New York

Statistics software

Analyses were done with SPSS versions 10 and 11, and the EQS program.

Radioimmunoassays

Antibodies: Scottish Antibody Production Unit

Other reagents: Amersham

Gamma counter: LKB Wallac 1260

Chapter 1: General Introduction

Individuals differ widely in how their cognitive ability changes with ageing (Christensen et al., 1999; National Research Council, 2000). Some develop the severe loss of cognitive ability seen in the dementias, others show milder but functionally significant degrees of cognitive impairment, and some exhibit very mild, if any, decline in some abilities. These variations are important, as those with relative preservation of cognitive function have a higher life expectancy and a higher quality of life (Korten et al., 1999).

A current major focus of research is the search for biological correlates underlying these important variations. Much of this work is aimed at discovering risk factors for the dementias, but there is also a substantial effort to determine the correlates of milder forms of cognitive impairment. In the last two decades various neuroimaging techniques have grown into a key part of the search for biological correlates of ageing-related cognitive decline, both in terms of an as yet unsuccessful method of reliably diagnosing the dementias (Scheltens et al., 2002), and in basic research.

Elevated levels of glucocorticoid hormones have been hypothesised to cause neuronal dysfunction, damage and eventual death, and to be an important cause of cognitive impairment in older people (Seckl and Olsson, 1995). However, there are many unresolved issues and few large studies in humans.

In this introductory chapter the issues involved in the measurement and characterisation of cognitive ability will be discussed, followed by a general survey of the literature on cognitive ageing. The background to the glucocorticoid hypothesis of cognitive ageing is surveyed and other putative risk factors for ageing-related cognitive decrements are also briefly reviewed.

1.1 General issues in the study of cognitive abilities

1.1.1 Definitions

There are many overlapping definitions of the term “cognition”. Common to most is that cognition involves the storage and manipulation of information by the brain.

Under this broad definition terms such as ‘reasoning’, ‘problem-solving’, ‘memory’, and so on, can be subsumed. In this thesis, operational definitions are used wherever possible.

The concept of intelligence is related to cognition but is more involved with the study of individual differences in cognitive abilities. Traditionally, delayed memory is not part of tests used to assess intelligence. The term ‘cognitive ability’ is broadly similar to intelligence, but implies the inclusion of a broader range of tests, for example tests of memory, than the term ‘intelligence’ has traditionally implied.

1.1.2 Approaches to the study of cognitive function

As in many branches of the behavioural sciences, the study of cognitive function has evolved into different domains or schools of thought, each with its own collection of assumptions, experimental approaches, database and theoretical foci. Perhaps three overlapping approaches can be identified: psychometric, neuropsychological and cognitive / experimental (Table 1.1). These approaches are becoming increasingly intertwined but important differences remain.

Table 1.1: Approaches to the study of cognitive function

Approach	Focus	Example of test
Psychometric	Individual differences in cognitive ability; structure of these individual differences. Emphasis on partitioning of variance, especially common variance	IQ (intelligence quotient) tests, memory tests
Neuropsychological	Individual differences in cognitive ability explicitly related to disorders of the brain. Emphasis on functional modularity.	IQ tests, memory tests, tests of 'executive function'
Cognitive/Experimental	Investigation of 'elements' of cognitive function, previously with little reference to the brain. The recently developed subdivision of cognitive neuroscience is more concerned with mapping of cognitive functions to particular brain structures/systems and this overlaps with the neuropsychological approach.	Tests exploring the elements of constructs like 'working memory', more recently, fMRI studies of brain activation during cognitive tasks

Psychometric

The psychometric approach is largely concerned with the science of measurement of differences in cognitive ability, and the determination of the covariance structure of cognitive abilities. This approach grew out from the development of intelligence quotient (IQ) testing more than one hundred years ago and is still best exemplified by the development of IQ-type tests and increasingly sophisticated methods of understanding the underlying structure of performance on various cognitive tests, in particular structural equation modelling (Deary, 2001a).

A simple form of this question is as follows: how do performances on different cognitive tests relate to one another? For example, is a person's performance on a test of list-learning independent of his or her performance on test of visuospatial problem-solving?

A major finding of psychometric psychology, first established by Spearman in 1904 (Spearman, 1904), is that performance on diverse cognitive tests correlates positively. Factor analytic techniques have been used to summarise these data, and have virtually universally found that the data are best summarised as a hierarchy of increasingly general sources of test variance with a general factor accounting for about 40-50% of the total test variance (Deary, 2001a). This general factor is commonly termed 'g', for general intelligence (Carroll, 1993). This *g* factor has several features which have added to its interest as an important variable and not just a statistical abstraction. *g* appears to have validity as a positive predictor of academic success, occupational success, health, and other real-life outcomes (Brody, 1992; Jensen, 1998; Mackintosh, 1998). *g* is relatively stable, in that a person tends to perform at around the same level when re-tested in the short term, and even several decades later (Crawford et al., 2001; Deary et al., 2000b).

Two other features of this approach must be noted. First, the vast majority of studies using the psychometric approach are on participants with brains which are largely intact, ie. in healthy populations. Second, the psychometric approach is essentially concerned with description and classification and no assumptions are made regarding the neurobiological apparatus underlying the different tasks, though there are many (largely speculative) correlative studies in the literature.

Neuropsychological

The neuropsychological approach has grown out of the clinical need to quantify the cognitive deficits occurring in the context of various injuries or diseases affecting the brain. The main principles in this approach are that different cognitive functions are performed by relatively discrete parts of the brain and that damage to particular brain structures is thought to cause predictable and quantifiable changes in performance on particular cognitive tests. As Sergent puts it, “More than a century of research has firmly established that the brain is organized into distinct areas of relative functional autonomy and specialization, and that local cerebral damage results in selective impairment of mental functions” (Sergent, 1988, quoted in (Salthouse, 2001a)). A list of the cognitive domains under study would include attention, language, memory, spatial abilities, executive function, working memory and general intellectual functioning (Lezak, 1995; Woodruff-Pak, 1997; Boller and Cappa, 2001).

In humans the hippocampus in the medial temporal lobe is known to be one of the structures essential for the storage and recall of factual information (Squire et al., 1993). One of the key advances in this field was provided by the study of a patient with intractable epilepsy, HM, who had both medial temporal lobes excised for the treatment of intractable epilepsy (Scoville and Milner, 1957). HM could then no longer store or recall new factual information such as a list of words read to him just minutes before. Thus his performance on delayed memory tasks is catastrophically poor. However, his performance on tasks not requiring the delayed recall of information was unaffected. He was even able to develop a high degree of skill in certain motor tasks, such as mirror-drawing (though he had no conscious awareness of having practised these skills before). In addition, his scores on IQ tests appeared to be largely unaffected. This led to the distinction between procedural and declarative memory and also to the conclusion that medial temporal lobe structures are essential for the normal functioning of declarative memory (Barba and Rieu, 2001).

Traditionally, the neuropsychological approach is orientated towards clinical practice, such as in neurosurgery and in memory clinics. Most of the data informing

this approach are thus derived from studies of patients with brain injuries or severe brain diseases such as the dementias. The variations in performance tend to be much larger than those seen in health or in milder, more diffuse disease processes. A memory deficit described by a neuropsychologist may be almost absolute, as in the case of HM.

Neuropsychologists and psychometrically-orientated psychologists use similar tests but the database, theoretical framework and goals are not identical. A crude analogy might be that a neuropsychologist is like a physiotherapist documenting why a person who has had a stroke cannot walk whereas a psychometrician is like an exercise physiologist studying the variables involved in determining the variance in a group of university students' speed over 100 metres.

Cognitive / Experimental

The cognitive approach is broadly concerned with understanding how the brain processes information; another way of putting this would be teasing apart the cognitive 'elements' involved in cognitive activities such as delayed memory. In the definitive early textbook, Neisser characterised cognitive psychology as, "refer[ing] to all processes by which the sensory input is transformed, reduced, elaborated, stored, recovered and used." (Neisser, 1967). For example, cognitive psychologists are interested in the multiple cognitive processes involved in reading.

A prominent example of this approach is the study of working memory, a construct first proposed by Baddeley and Hitch (Baddeley and Hitch, 1974). According to Baddeley and Logie, "working memory comprises multiple specialized components of cognition that allow humans to comprehend and mentally represent their immediate environment, to retain information about their immediate past experience, to support the acquisition of new knowledge, to solve problems, and to formulate, relate, and act on current goals." (Baddeley and Logie, 1999). There is a variety of models of working memory, but most involve a central executive with two associated

‘slave’ systems, a visuospatial sketchpad and a phonological loop. The phonological loop is the theoretical subsystem which is responsible for maintaining in mind for brief periods small pieces of information such as a telephone number, which decay rapidly without rehearsal. The central executive is brought into play when such information has to be manipulated in some way. There is currently much debate as to the exact configuration of working memory, which is perhaps unsurprising given the sheer amount of cognitive work it is hypothesised to subserve (Shah and Miyake, 1999).

Increasingly, with the growth of technologies such as functional magnetic resonance imaging, cognitive psychologists are also interested in discovering what structures and systems in the brain are necessary for a certain task. This has led to the birth of the discipline of cognitive neuroscience, which, “... aims to understand how cognitive functions, and their manifestations in behaviour and subjective experience, arise from the activity of the brain.” (Rugg, 1997).

1.1.3 The classification of cognitive abilities: the hierarchical model

A glance through a selection of the major neuroscience and psychology journals quickly informs the reader that there is a confusion of terms and constructs in the study of cognitive ability. Researchers use terms like short-term memory, executive function, processing speed, attention, frontal lobe function, hippocampal function, and so on. It is an interesting feature of psychological research that few acknowledge that these terms represent a loose collection of ill-supported and ill-defined constructs rather than well-established and quantifiable natural phenomena with parallels in the natural sciences. In fact, there is no classification of cognitive abilities which is in use for the majority of researchers studying cognitive abilities.

From an empirical perspective, one of the most consistent findings in any field of cognitive ability research is that virtually all cognitive tests positively intercorrelate.

Perhaps the clearest demonstration of this has been provided by Carroll, who collected the data from 450 studies (selected for size and in which diverse tests had been used) and subjected these data to factor analysis, by the same procedures for each dataset (Carroll, 1993). Carroll found that all the tests intercorrelated positively. That is, tests on apparently very different tasks, such as recalling geometric shapes or knowing the correct pronunciation of irregularly-spelt words, vary together. Typically the covariance among a battery of cognitive tests ranges from 40-60%, that is, 40-60% of the variance is shared among the tests.

Performance on tests which are more similar to each other (eg. tests of abstract reasoning) correlate more closely than with different types of tests (eg. tests of verbal memory), though the correlation with *g* is usually higher. Because of this, Carroll proposed the three-stratum model of cognitive abilities. At the top of the hierarchy is *g*. In the second stratum is a variable number of group factors. The second stratum factors are labelled according to the type of tests which tend to correlate more closely together. In Carroll's model these factors are labelled fluid intelligence, crystallised intelligence, general memory and learning, broad visual perception, broad auditory perception, broad retrieval ability, broad cognitive speediness, and processing speed (Carroll, 1993). There are other versions of the model, with fewer second stratum factors; differences in versions of the model depend partly on the tests used and the type of factor analytic procedures used. The third stratum is made up of the individual test scores. Factor analysis and structural equation modelling studies have supported the validity of this structure, and have also repeatedly refuted the main alternative hypothetical structure of statistically separate cognitive components.

The hierarchical model finds that a raw test score results from a combination of general variance, group factor variance, test-specific variance, and other (error) variance. This is a very important finding because of the widespread use of cognitive tests. However, the influence of *g* on an individual test score is often not taken into account.

Many researchers question the existence of *g* and/or the hierarchical model. Objections have been made on various grounds, including methodology, with

criticisms directed at the selection of samples, and the use of factor analysis; the conceptual basis *g* - for example, how can a brain thought to be composed of functionally distinct modules give rise to a large general factor?; the social undesirability of IQ; and the associations of a minority of IQ researchers with eugenics (Gould, 1981; Neisser et al., 1996; Montagu, 1999). Interestingly, there are very few empirically-based refutations of the existence of *g*, in large-scale studies, at least.

Perhaps the best-known semi-popular work arguing against a general factor in intelligence is Howard Gardner's *Frames of Mind* (Gardner, 1993). In this book Gardner puts forward the argument that there are 'multiple intelligences' which are independent in each person. These are: visual/spatial, verbal/linguistic, mathematical/logical, bodily/kinaesthetic, musical, intrapersonal, interpersonal. Of these the first three are in the generally accepted area of study of cognitive ability research. Although these ideas make intuitive sense, no empirical evidence has emerged to support the idea that performance on these visual/spatial, verbal/linguistic and mathematical/logical domains varies orthogonally (that is, showing no correlation). However, there are clearly parallels between these 'intelligences' and second-order factors commonly identified in the hierarchical model.

Some neuropsychologists continue to question the ideas of *g*, or at least its interpretation, and the hierarchical model (Das, 2002; Dodrill, 1997; Larrabee, 2000; Pallier et al., 2000; Ardila et al., 2000; Ardila, 1999; Tremont et al., 1998b; Larrabee et al., 1983; Woodruff-Pak, 1997). For example, Woodruff-Pak has claimed that, "Intelligence tests do not really belong in the category of neuropsychological assessments, as the global IQ score is not associated with discrete brain function." (Woodruff-Pak, 1997). Examples of case studies showing clear dissociations between cognitive domains are often given, with HM as the prototypical example. These dissociations do demonstrate that the hierarchical model in its original form does not apply to every conceivable circumstance. In particular, where there is discrete damage, performance on certain tests (which may or may not correspond to

a group factor) might show dramatic decline. However, this does not mean that in healthy populations the hierarchical model is invalid.

Objectors often confuse the concept of *g* as an empirical reality with the assumption that believers in *g* favour a monolithic explanation of brain function. However, the idea that the brain is composed of an unknown number of modules performing different processing tasks is not incompatible with idea of *g*. *g* might reflect important bottlenecks in the process, or there may be genetic or biological effects that affect a large proportion of the processing modules (Anderson, 1992). In addition, only 50% of the variance of a battery of cognitive tests is shared; the group factors represent more specific clusters of variance and thus the hierarchical model can readily accommodate the notion of theoretical modules or clusters of modules (Deary, 2001a). As stated above, the psychometric approach is primarily concerned with the accurate measurement and classification of cognitive function and not with determining the underlying brain processes and structures responsible for cognitive function.

1.1.4 Memory

Although performance on memory tasks is incorporated into the hierarchical model of cognitive ability, memory has a prominent place in cognitive ageing research and a brief overview of the field of memory research is presented here. Cognitive and neuropsychological research over several decades in animals and humans has yielded a broad consensus on a behavioural model of memory. Performance on memory tasks is reliably separable with respect to the lengths of time involved, and also in the type of information that is being stored. In terms of time, short-term memory can be distinguished from long-term memory. Amnesic patients are characterised by their inability to register and/or recall information beyond periods of a few seconds or minutes, but commonly show normal performance on when asked immediately to reproduce short strings of letters or numbers or other material (Cave and Squire,

1992). Working memory is a construct which is related to short-term memory; it is discussed below.

Long-term memory involves retaining information beyond a few minutes. It is separable into "... conscious memory for facts and events and various forms of nonconscious memory, including skill and habit learning, simple classical conditioning, the phenomenon of priming, and other instances where memory is expressed through performance rather than by recollection." (Squire et al., 1993). Squire has called the conscious memory for facts, "declarative memory", and other types of memory "nondeclarative memory". This declarative versus nondeclarative dichotomy has been given other labels, such as explicit versus implicit.

A model of memory in humans which has gained widespread acceptance is that of Schacter and Tulving (Schacter and Tulving, 1994). This is displayed in simplified form in Table 1.2.

Table 1.2: Schacter and Tulving's model of human memory (simplified)

System	Other terms
1. Procedural	Non-declarative
2. Perceptual representation	Non-declarative
3. Semantic	Generic, Factual, Knowledge
4. Primary	Working memory, Short-term memory
5. Episodic	Personal, Autobiographical, Event memory

This model divides declarative memory into primary, semantic and episodic categories. Semantic memory refers to general knowledge about the world and episodic memory to events with a specific context in space and time. This distinction has been made on the basis of the study of patients with different types of dementia, in which, to some extent at least, some patients with severely impaired episodic memory can acquire semantic knowledge, and vice versa (Squire et al., 1993; Hodges and Graham, 2001).

In animals, parallels to the declarative versus non-declarative model can be found. In the Morris water maze (Morris, 1981), rats are placed in a circular tank of water with sheer walls and swim until they find a submerged platform which they mount. The tank is in a room with some spatial cues on the walls, providing the rats with spatial information. Rats learn in a few trials where the platform is and this can be measured by the time from placement into the tank to mounting of the platform. Lesions to the hippocampal system in rats and other animals severely disrupts or abolishes this type of learning, but does not abolish simple classical conditioning, priming, or other types of learning analogous to nondeclarative learning in humans. Thus, a broad parallel between declarative memory in humans and spatial memory in animals has been drawn (Cohen and Eichenbaum, 1994).

Research on memory has mainly been orientated towards describing the general cognitive processes involved in memory tasks and the brain structures and systems involved in these processes. Most of the individual differences research on memory and *g* has focused on short-term memory and working memory, in which positive associations with *g* are consistently found (Carroll, 1993; Conway et al., 2002). However, less is known concerning performance on tests of delayed memory and *g* in healthy populations. What research there is has shown positive associations, as predicted by the hierarchical model (Salthouse, 2001a).

In the next section, sample studies from the literature on the relationships among diverse cognitive tests are summarised.

1.1.5 General cognitive ability and performance on neuropsychological tests

In this section, the term ‘neuropsychological tests’ refers to tests which are used to assess memory and other ‘specific’ cognitive abilities, as distinct from tests thought to measure general cognitive ability.

Dozens of functions have been attributed to the frontal lobes, including working memory, executive function, planning, reasoning, problem-solving, temporal organisation of behaviour, selective inhibition of responses to immediate memory, monitoring of function, and so on (Ardila et al., 2000; David, 1992). Amongst most neuropsychologists the prevailing view has been that there is little relationship between performance on standard tests of intelligence and tests of the various constructs attributed to the frontal lobe listed above (Duncan et al., 1997; Damasio, 1985), at least in situations in which there is damage to the frontal lobes. Recently, however, there have been increasing efforts to examine performance on tests of these constructs in the context of general cognitive ability.

Frontal lobe function

Obonsawin and colleagues (Obonsawin et al., 2002) examined performance on several tests of ‘frontal lobe’ function (verbal fluency, Modified Card Sorting Test, Stroop test, Tower of London, cognitive estimates, paced auditory serial addition task, and uses of common objects task) in relation to performance on a standard test of IQ, the Wechsler Adult Intelligence Scale-Revised (WAIS-R). The subjects were 123 healthy individuals recruited from the community. All of the frontal lobe tests correlated significantly with WAIS-R scores in the predicted directions at between 0.24 and 0.63. The intercorrelations among the frontal lobe tests were virtually all significant. When these intercorrelations were controlled for WAIS-R scores very few significant correlations remained. This suggests that much of the shared variance among the frontal lobe test scores is due to general cognitive

ability and not a specific factor reflecting frontal lobe functioning. Principal components analysis of the frontal lobe test scores yielded two factors accounting for 53% of the variance; all but the Modified Card Sorting Test scores loaded on the first factor. Interestingly, loadings on the first correlated at 0.72 with WAIS-R scores. The authors interpreted their findings as showing that in a healthy population, general cognitive ability is the single most important determinant of performance on tests designed to measure 'frontal lobe function'. Further doubt on the labelling of 'frontal lobe' tests as such has emerged with functional magnetic resonance imaging (fMRI) studies on subjects performing these tasks. Several of these studies have found that during the performance of tasks such as card sorting and the Tower of London several parts of the brain show activity, including the basal ganglia, thalamus and cerebellum (Cabeza and Nyberg, 2000).

Working memory

Working memory is a construct that is characterised as an active system for temporarily storing and manipulating information needed in the execution of complex cognitive tasks (Baddeley and Wilson, 2002; Bright and Kopelman, 2001). The working memory model incorporates a central executive served by two systems, one holds verbal and acoustic information and the other visuospatial information; recently Baddeley proposed the existence of another system: the 'episodic buffer', which combines information in working memory and long-term memory into a single representation (Baddeley, 2000). Studies of working memory have largely been of the cognitive/experimental and neuropsychological approaches. Until fairly recently working memory was not part of the vocabulary of psychometric psychologists. Recently, there is a growing number of studies which have examined correlations among individual differences in working memory tasks and measures of general cognitive ability.

Larson and Saccuzzo (Larson and Saccuzzo, 1989) found a correlation of 0.5 between a working memory task called 'mental counters' and performance on

Raven's Standard Progressive Matrices, a standard test of intelligence. In 1990 Kyllonen and Christal found correlations of 0.45 to 0.58 (these were all statistically significant at $p < 0.01$) between working memory tasks and tests of reasoning, which are known to correlate highly with g (Kyllonen and Christal, 1990). The correlations among the latent traits were even higher. They suggested that their results are supportive of the hypothesis that individual differences in "working memory capacity [are] primarily determined by individual differences in reasoning ability."

Engle and co-workers (Engle et al., 1999) asked 133 subjects to perform nominally different types of tests: working memory tests, which they had selected as being the best examples in the field, short-term memory tests including forward and backward word-span, and fluid intelligence (Gf), using Raven's Standard Progressive Matrices and the Cattell Culture Fair test. Performance on the working memory and short-term memory tasks was correlated but structural equation modelling indicated that the best model did indeed have separate factors for working memory and short-term memory. The overall model showed that factor representing the shared variance between the working memory factor and short term memory factors correlated at 0.29 with the Gf factor (derived from the Raven's Standard Progressive Matrices and the Cattell Culture Fair test), and the specific relationship between the working memory factor and Gf was 0.49. These results suggest that the variance on the working memory tests closely corresponds to variance on the fluid intelligence tests.

Memory

Most studies looking at dementias and other forms of cognitive impairments will include tests of memory in their batteries. These tests tend to involve presentation of lists of words or pictures of shapes followed by requests that the subject recall the material immediately and at defined intervals. As indicated above there is a consistent positive relationship between the two constructs, but surprisingly studies which measure both rarely measure this correlation.

Rapport and co-workers (Rapport et al., 1997) studied the relationship between IQ as measured by the WAIS-R and several memory tests, including immediate and delayed paragraph recall and list-learning. Tests were administered twice, on sessions two weeks apart. The Verbal Comprehension and Freedom from Distractibility scores on the WAIS-R explained 42% of the variance in the memory scores. Full scale IQ from the first session explained between 22% and 41% of the variance in memory scores in the second session. A similar pattern was reported by Salthouse and co-workers (Salthouse et al., 1996), who studied performance on a variety of tests in 259 healthy subjects aged 18 to 94. They found multiple significant positive intercorrelations, for example the Wisconsin Card Sorting Test, traditionally a test of executive function, correlated at $r=0.49$ with the WAIS-R Block Design subtest and at $r=0.27$ with the Rey Auditory-Verbal Learning Test (a test of declarative memory).

Keenan and co-workers (Keenan et al., 1996b) analysed the relationship of the WAIS-R Vocabulary scale to performance on a widely-used test of declarative memory, the California Verbal Learning Test. Subjects were 82 healthy adults aged 16-95. In hierarchical multiple regression analyses Vocabulary scores accounted for 31% of the variance in memory scores. These studies are in keeping with the hierarchical model and suggest that researchers cannot ignore the effects of general cognitive ability when interpreting memory scores, particularly in relatively healthy populations.

1.1.6 Conclusions

Researchers studying cognition have generated a large number of terms to describe aspects of cognitive function. Many of these terms have face validity; that is, it seems reasonable to write about 'executive function' and 'speed of processing'. However, quite apart from frequent semantic overlapping, there is also the issue of what empirical evidence there is to support the existence of a cognitive construct. As Deary states, "Construct validity ... tell[s] us that there is only as good evidence for the existence and usefulness of a construct as there is empirical evidence." (Deary, 2000)(p.101). Many of the terms in widespread use fall short of having achieved construct validity.

For example, the term "hippocampal function" is in common use in studies on memory in humans (Collie et al., 2002; Small et al., 2002). This term is sometimes used interchangeably with constructs such as delayed declarative memory. However, although certain memory tasks probably depend on a functioning hippocampus, the extent to which performance on these tests actually reflects individual differences in the functioning of the hippocampus is unknown. Intuitively, performance on most cognitive tests would seem to depend on the correct functioning of several hypothetical cognitive functions or operations involving multiple unknown brain systems. For example, in an ostensibly simple test of paragraph recall, the subject is required to maintain attention on task instructions, understand these instructions and keep them in mind, attend to the paragraph being read, retain this information, and so on. Given the complexity and number of these cognitive operations it is hard to see how, in absence of focal brain damage, one can confidently assert that the individual differences in the function of the hippocampus is the only or even the most important neurobiological variable and that this is reflected in test performance which is orthogonal to other performances on other tests. This intuitive objection is borne out empirically by the massive weight of evidence underlying the hierarchical model.

Some mapping of structure to function is possible when there is severe damage to a structure; the best example of this is the link between hippocampal damage and

deficits in declarative memory. Here there is not only focal brain damage but also a change in cognitive performance which is considerably more severe than the types of ranges seen in healthy populations. Therefore, the structure of the cognitive abilities as described in the hierarchical model does not apply in all circumstances.

With most populations, however, cognitive tests show positive intercorrelations, and failing to take this into account could lead to misinterpretation of cognitive test data. This is a problem which might be particularly important in circumstances in which cognitive change is relatively diffuse and/or subtle. This is a common scenario in research in cognitive ageing, which is covered in the next section.

1.2 Cognitive function and ageing: descriptive studies

1.2.1 Introduction

Cognitive function changes with ageing. This is an extremely complex research issue because (1) these changes occur on the background of individual differences in cognitive abilities, (2) different abilities might change at different rates and with different trajectories, (3) there are individual differences in the extent to which these abilities change. Underlying some of these changes are universal changes in the nervous system as well as the emergence of several more specific disease processes with increasing age, which affect cognitive abilities in different ways.

The literature reflects not only the complexities outlined above but also the different theoretical perspectives of the researchers. A variety of models, some stated more explicitly than others, influence the selection of tests, subject populations and interpretations of results. An important divide is between studies which simply document the scores on various tests across age ranges or across time and studies which attempt to take account of pre-morbid functioning and/or use statistical techniques to look for more parsimonious explanations in terms of latent traits.

Studies also differ in terms of their aim. Some are concerned with the natural history of various cognitive abilities with ageing, whilst others are focused on identifying patterns of cognitive function which are predictive of dementias or which are characteristic of dementias.

In this section of the introduction, an overview of study designs and concepts in cognitive ageing research is provided, and then some of the key empirical literature is reviewed.

1.2.2 Study designs

Studies on humans of cognitive function in ageing are cross-sectional, longitudinal or a combination of cross-sectional and longitudinal, cross-sequential (Deary, 2000; Schaie and Hofer, 2001). Most studies are cross-sectional, using either a narrow range of ages and comparing performance to 'norms' for the tests concerned, or using a range of ages and examining the effects of age. The main problem with this method is cohort effects, that is, older subjects have a different cultural and educational history than younger subjects and it is impossible to attribute differences in cognitive function to age alone. These studies are also affected by the finding that IQ scores have risen in real terms over the past few decades (Flynn, 1987).

The next major category is longitudinal, in which a cohort of subjects is examined at intervals over time. These studies are affected by several problems, including practice effects, in which subjects' performances on tests improves with each wave of analysis, non-random attrition, in which the survivors, particularly in older age groups, tend to be healthier. Also, cohort and period effects affect interpretation, that is, a group people born and developing in a certain period in history are subject to a combination of biological, social and cultural influences which are unique to that group of people (Schaie and Hofer, 2001). Prominent examples of this type of study include the Nun Study (Riley et al., 2002) and the MacArthur Studies of Successful Aging (Chodosh et al., 2002).

The third main category is cross-sequential. This is a combination of cross-sectional and longitudinal designs. An initial cohort is recruited, examined and followed up as in the case of standard longitudinal designs. However, further cohorts are recruited at each wave of examination, allowing for both cross-sectional and longitudinal analyses, as well as allowing the researcher to examine cohort effects. The best example is the Seattle Longitudinal Study (Schaie, 1994), which began in 1956 and has involved over 5000 subjects. Initially, 500 subjects aged between 20 and 90 had their cognitive ability tested with a set of well-validated tests from Thurstone's primary mental abilities battery. These subjects were followed up every 7 years, with

between 600 and 1000 new subjects from across the age range being recruited and tested at each wave. This study has yielded important data on cognitive changes with ageing, and also has demonstrated strong cohort effects: the most recently-recruited cohort showed significantly better mean cognitive performance than the first cohort (Schaie, 2000).

1.2.3 Concepts in cognitive ageing research

Salthouse (Salthouse, 2001b) recently posed the question of how one might explain the well-documented age-related effects on a measure of free recall memory. Are there “age-related effects on processes specific to free recall [?]” Or might there be general effects of ageing affecting many different tasks, including free recall tasks? As with general research on individual differences in cognitive function, psychometric and neuropsychological approaches to cognitive ageing research can be distinguished. The neuropsychological approach is based on the premises that, “... age-related effects on different variables are attributable to discrete effects on specific neuroanatomical structures, and each compromised function should ultimately be traceable to a deficit in the structure or region specialized for that function.” (Salthouse, 2001a). The psychometric approach is concerned with describing and analysing the covariance among cognitive tests in an attempt to discover possible latent variables which explain the patterns observed.

Inhibition

A construct in common use in the cognitive ageing literature is that of inhibition. This can be defined as, “... the ability to attend selectively to goal-related signals and to ignore, and withhold (“inhibit”), habitual, or over-practised responses to other stimuli ...” (Rabbitt, 1997). The frontal theory of cognitive ageing is based on the two basic premises that the frontal lobes show the greatest decrements in volume with ageing, and that this is reflected in performance on ‘frontal’ tasks (West, 1996).

Most other studies with the neuropsychological approach are focused on declarative memory in relation to decrements in medial temporal lobe structures, particularly the hippocampus. These studies are often aimed at attempting to predict individuals at risk of dementia. Most studies centring on inhibition and/or memory do not address the issue of possible shared variance among tests, or the influence of baseline cognitive ability on test performance.

Fluid and crystallised intelligence

Two of the most important concepts in the psychometric approach are the distinction between fluid and crystallised intelligence, and the role of processing speed. The ideas of fluid and crystallised intelligence were originated by Cattell in the early 1960s. Cattell's theory of intelligence (Cattell, 1963) is based on the idea that *g* is separable into two components, fluid intelligence (*Gf*) and crystallised intelligence (*Gc*). Cattell hypothesised that *Gf* was biologically determined and showed decline over the adult lifespan and that *Gc* reflected knowledge acquired over the lifespan and did not show decline (Brody, 2000). Currently, *Gf* is seen as being equivalent to fluid, abstract reasoning and *Gc* as being broadly equivalent to overall knowledge (Mackintosh, 1998). This theory predated Carroll's factor analytic survey; in that framework *Gf* and *Gc* are first two second stratum factors, and are the two which are most closely correlated with *g*.

Gf and *Gc* are very highly correlated in young adulthood, but start to show lower correlations with ageing (Schaie, 1994). The *Gf/Gc* split in terms of ageing in relatively healthy populations has broadly been supported by numerous large studies (Kaufman, 2001). Some of these studies are reviewed below. However, there is a paucity of studies applying these constructs to the prediction of dementia (Deary et al., 1998) and little is known about their utility in that context.

Processing speed

Related to the Gf/Gc model of cognitive ageing is Salthouse's processing speed theory of cognitive ageing (Salthouse, 1985). This is centred on the proposal that age-related decrements in the construct 'processing speed' account for much of the variance in any age-related cognitive decline. Processing speed has been measured with numerous psychometric tests whose score depends on speed of response and/or speed of activity, such as the Digit-Symbol Substitution Test of the WAIS. The implication is that the effects of ageing are mediated through a common mechanism or set of mechanisms rather than there being specific age-related effects for each cognitive domain.

Numerous studies using multiple regression and structural equation modelling techniques have indeed confirmed that performance on tasks involving a timed element and/or problem-solving with novel information shows much shared variance which more-or-less accounts for the effects of age. However, the nature of processing speed remains unclear and the original hypothesis that this shared variance was all due to processing speed has undergone some modification. Recently Salthouse and others have concluded that the nature of this shared variance is perhaps not best defined as specifically as 'processing speed' but is perhaps more closely aligned with the construct of Gf (Deary, 2000; Salthouse, 2001b).

1.2.4 Overview of the literature

In this section studies drawn from various approaches will be reviewed. It is difficult to classify the different types of studies because of their different conceptual bases and aim. Therefore the studies reviewed are only loosely grouped according to the main focus of the enquiry. The studies are selected on the basis of size and aim and are intended to be a sample of the literature rather a systematic review.

The long-term stability of individual differences in cognitive ability

There are few studies which have looked at the long-term stability of individual differences in cognitive ability. These studies are rare mainly because of the long time periods involved.

Deary and co-workers recently discovered records from the Scottish Mental Survey of 1932, in which virtually all Scottish schoolchildren born in 1921 had their intelligence measured using the IQ-type Moray House Test, which correlates at 0.80 with the Stanford-Binet standard test of intelligence (Deary et al., 2000b). A total of 87,498 children took the test. 101 of these individuals were identified and administered the same test at the age of 77. Performance on these tests correlated at 0.63, 66 years apart. Correction for restricted range increased the strength of the correlation to 0.73. This study provides the longest follow-up period of any such study and is the only one which used identical tests. Clearly, the stability demonstrated here was in surviving, healthy individuals; it cannot be known from this study whether good health is a determinant of relatively greater stability in intelligence.

Plassman and co-workers (Plassman et al., 1995) performed a 50-year follow-up of cognitive function in 930 men who had been administered the Army General Classification Test as part of the general evaluation process for new recruits in the Second World War. This test correlates highly (0.70 – 0.90) with standard tests of intelligence. They did not administer this test again, but used a modified form of Telephone Interview for Cognitive Status, a brief telephone-administered test often used to screen for dementia. The two tests correlated at 0.46.

Studies focusing on IQ

The following studies have used IQ-type tests in the context of ageing. That is, other than the literature underpinning the construct validity and reliability of IQ, assumptions were not made about the biological mechanisms involved in any

changes observed. However, IQ tests are typically made up of several subtests, and several investigators have attempted to examine possible differences in the impact of ageing on different subtests or clusters of subtests, in particular those thought to reflect Gc versus Gf.

Korten and co-workers studied cognitive function on two occasions three and a half years apart in 1135 subjects aged between 70 and 102 years (Korten et al., 1997). They used four tests. The National Adult Reading Test (NART) was used as a measure of crystallised intelligence. The Mini-Mental State Examination (MMSE) is a broad cognitive screening test including items on memory, orientation, language, and other cognitive domains. It is scored out of 30; in healthy subjects scores range from 27-30 with most scoring 30, thus there is a strong ceiling effect. The Symbol Letter Modalities Test is designed to measure 'cognitive speed'. Subjects are asked to read out letters corresponding to geometric symbols as quickly as possible. Finally, the Episodic Memory Test, which consists of three short memory tasks: three-word recall, name and address recall, figure reproduction and face recognition. NART scores showed no change over this period. MMSE scores showed small but significant declines despite clear evidence of a ceiling effect. The Episodic Memory Test, perhaps surprisingly, showed an improvement overall, though the older subjects showed decline. The authors attributed any improvements partly to learning effects. The Symbol Letter Modalities Test showed significant decline across the age range. This study showed the commonly-observed pattern of clear decrements in a test requiring rapid responses but no decrements in crystallised ability.

Ryan and co-workers examined age effects on Wechsler Adult Intelligence Scale III (WAIS-III) subtests (Ryan et al., 2000). The IQ score from the WAIS and other intelligence tests reflects a composite of scores from 14 subtests, adjusted for age. On the basis of factor analysis these are traditionally divided into two groups, named Performance and Verbal subtests. This study examined data from the WAIS-III standardisation sample. This has 13 age groups with about 100 males and 100 females in each group. The WAIS-III was administered to each participant. Scores on five (Vocabulary, Similarities, Arithmetic, Information and Comprehension) of

the seven Verbal subtests did not show declines with age, apart from in the two age group bands above the age of 79. Scores in these five subtests tended to show small improvements from the age of 30 to 80. Scores on the Digit Span and Letter-Number Sequencing subtests showed small declines from the 45-54 age group. By contrast, scores on all seven of the Performance subtests (Picture Completion, Digit Symbol, Block Design, Matrix Reasoning, Picture Arrangement, Symbol Search and Object Assembly) showed significant declines from the 45-54 age group onwards. Although the WAIS III subtests are not specifically designed to measure fluid or crystallised intelligence, scores on certain Performance subtests (such as Matrix Reasoning) are often assumed to be linked with Gf and certain Verbal subtests (such as Vocabulary) with Gc. This notion holds true for 12 of the 14 tests in this study.

Many other studies have confirmed this general pattern with the WAIS subtests. Another large cross-sectional study was conducted by Kaufman and Horn (Kaufman and Horn, 1996). They used the Kaufman Adolescent and Adult Intelligence Test (KAIT), a cognitive battery with three subtests designed to measure Gc and three to measure Gf. They studied scores on this test in a sample of 1500 men and women aged 17-94. The age range was divided into 13 groupings. They found that the measures of Gf peaked in the second youngest group (20-24) and showed steady decline from that point onwards, with the rate of decline accelerating from the 50-54 age group onwards. The Gc subtests were stable until the 60-64 age-group, at which point there was small decline in each age group. This decline was smaller than for the Gf subtests.

One of the largest studies of cognitive function and ageing is the Seattle Longitudinal Study, which was described above. The main findings from this study are that there are declines in tests of reasoning, verbal memory, perceptual speed and spatial orientation from the mid-twenties until age 81. In contrast, tests of verbal ability and other tests thought to reflect crystallised ability did not show decline until over age of 70 (Schaie, 1994; Schaie, 2000).

The above studies provide convincing evidence that certain cognitive abilities: those involving a timed element and/or the learning, retention and manipulation of new

information, decline with ageing, whereas those abilities involving prior learning show little or no decline.

Studies focusing on memory

The following studies have tended to examine scores on different cognitive tests as statistically separate entities and, because of the longstanding interest in the impact of ageing on memory, have focused on performance on tests of memory.

Park and co-workers (Park et al., 2002) subjected 350 adults across an age-range of 20 to over 80 to tests of working memory, short-term memory and delayed memory. They found that as expected, there is a steady decline in tasks requiring the manipulation or learning of new information, including working memory and long-term memory. Verbal knowledge increased with age.

In a longitudinal study Small and co-workers (Small et al., 1999) attempted to answer the question of “whether aging is associated with memory decline.” 212 community-based subjects aged 60-93 were tested annually on four occasions on a battery comprising tests of simple orientation, language (including verbal fluency, often described as a test of ‘executive function’), abstract reasoning (including the Similarities subtest of the WAIS), visuospatial ability and memory. The memory test involves subjects recalling a list of words immediately after presentation over several trials, and then after a fifteen-minute delay. To assess the effects of age in this sample subjects were stratified into two age groups using 70 as the cut-off point. They found that only performance on the verbal memory task showed an age group by time interaction, that is the older age group had a higher relative decline compared with the younger group. Interestingly, this effect was only observed for the non-delayed component of the test. Age group also had significant effects on the visuospatial tests and the Similarities subtest of the WAIS. Performance on many of the tests showed improvements with time, which authors interpreted as being due to practice effects.

Important weaknesses of this study were that no attempt was made to control for premorbid ability or other relevant constructs such as processing speed. Also, the authors did not look at intercorrelations among the different tests. Thus it is difficult to interpret the findings as they might represent baseline differences in overall cognitive ability, or declines in fluid intelligence and/or processing speed rather than specific deficits in memory. In this light, it is of note that only the non-delayed component of the verbal memory test showed the significant effects. This study is a good example of how failing to take account of latent traits in cognitive function makes interpretation of the findings difficult.

Vakil and Agmon-Ashkenazi (Vakil and Agmon-Ashkenazi, 1997) compared performance between young and older adults (two groups of 25, aged 21-35 and 60-84) on two tests of immediate and delayed verbal and visual declarative memory and two tests of procedural learning. They hypothesised that the older group would show the greatest relative deficits on the declarative memory tasks. In fact the older subjects were relatively impaired on both types of task, though the gap between the rate of learning of the items in the declarative memory task was greater than for the procedural memory tasks. Again, there was no attempt to control for baseline ability or other intervening variables.

The above studies, and many others besides, show that performance on memory tests involving the learning and recall of new information does tend to decline with ageing. Furthermore, there is some evidence that performance on declarative memory tasks might show greater decrements than performance on procedural memory tasks. However, the majority of these studies have not examined the effects of premorbid or peak overall ability, and have not tested the hypothesis that a decline in a latent trait representing Gf or processing speed might be largely responsible for the declines observed. Thus, many investigators have concluded that there is a relatively circumscribed decrement in the construct of declarative memory.

Studies focusing on frontal lobe function and working memory

As stated above, an important strand of the cognitive ageing literature is that looking at impact of ageing on various cognitive constructs thought to be linked with frontal lobe function. In this section some of the larger studies in this area are reviewed.

Ronnlund and co-workers (Ronnlund et al., 2001) examined performance on the Tower of Hanoi puzzle, the Block Design subtest of the WAIS, verbal fluency and a test of delayed declarative memory in relation to age. The subjects were part of the large Betula prospective cohort study (Nilsson et al., 1997), but the data are from one wave of analysis and are thus cross-sectional. There were 2798 male and female subjects aged from 35 to 85, in 11 groups aged (at the time they were tested) 35, 40, 45, and so on. There were significant age effects on Tower of Hanoi performance; however, these were almost eliminated by controlling for performance on Block Design, verbal fluency and the delayed declarative memory task. This study, one of the largest of its type, strongly suggests that tests involving active problem-solving/manipulation of information, not only decline with age but decline together. This adds support to the idea that changes in fluid intelligence underlie most changes observed in cognitive ageing.

Isingrini and Vazou (Isingrini and Vazou, 1997) compared performance on standard tests of intelligence, the WAIS Vocabulary, Information and Similarities subtests and Cattell's matrices with performance on widely used tests of frontal lobe function, the Wisconsin Card Sorting Test and two tests of verbal fluency. The aim was to test the hypothesis that in an elderly population there would be a positive correlation between fluid intelligence tests and frontal lobe tests. The subjects were 35 adults aged 25 to 46 and 72 adults aged 80 to 99. None of the subjects fulfilled criteria for dementia. The results were presented separately for each age group. In the younger age group modest but largely non-significant correlations were found in the predicted directions among frontal and intelligence tests. In the older age group the Similarities subtest from the WAIS and Cattell's matrices correlated at levels of 0.32 to 0.59 with the frontal tests. The authors interpreted these findings as demonstrating that there is

some overlap among tests of fluid intelligence and frontal lobe function, at least in older age groups.

Hanninen and co-workers wished to determine if patients with a formal diagnosis of age-associated memory impairment also had deficits on other types of cognitive function (Hanninen et al., 1997). In a cross-sectional study they compared performance on traditional tests of frontal lobe function (verbal fluency, the modified Wisconsin Card Sorting Test, the Trail Making Test and the Stroop test) in 43 patients who fulfilled criteria for 'age-associated memory impairment' with performance in 47 age-matched controls. They found that performance on all of these tests was significantly worse in patients versus the controls.

Schretlen and co-workers (Schretlen et al., 2000) wished to determine the relationships among individual differences in 'perceptual comparison speed', working memory, executive function, fluid intelligence and crystallised intelligence in adults aged between 20 and 92. MRI was used to determine frontal lobe volumes; these results are not considered in detail in this section. The test of perceptual comparison speed was developed by Salthouse and involved, "... the rapid comparison of letter strings (composed of 3 or 6 letters each) or line patterns (composed of 3 or 6 lines each). The participant was required to indicate whether the letter strings or designs that comprised each pair were identical or different by marking an 'S' or a 'D' in the space between them. The number of correct comparisons made in 30s was recorded in each task ...". Working memory was assessed using Schretlen's Brief Test of Attention, in which subjects listen to audio presentations of mixed digit-letter strings from 4 to 18 in length, and is asked to keep in mind the number of letters in each string. Executive function was assessed with the Modified Wisconsin Card Sorting Test. For the fluid and crystallised intelligence measures, a principal components analysis (with varimax rotation) was performed on a battery of further tests. This yielded a two factor solution accounting for 68% of the variance. The National Adult Reading Test, the WAIS-R Information, Arithmetic, and Similarities subtests loaded onto one, designated the crystallised intelligence factor (Gc). The Benton Facial Recognition Test, the Rey-Osterrieth Complex Figure

Test and the WAIS-R Picture Completion and Block Design subtests loaded onto the other, designated the fluid intelligence factor (Gf).

Age correlated at -0.56 ($p < .001$) with Gf; there was no correlation between age and Gc. Interestingly, working memory correlated with Gf at 0.51 ($p < .001$); other correlations among the tests and factors were not presented. Hierarchical multiple regression analyses were then used. With Gf as the dependent variable, using age alone accounted for 28% of the variance. The addition of terms for perceptual comparison speed and working memory increased the variance explained to 46%, but only the beta weight for perceptual comparison speed was significant. The analysis was repeated without the inclusion of age. Terms for perceptual comparison speed and working memory gave an equation explaining 45% of the variance; adding age at that stage did not improve the model. Neither executive function nor Gc were included in this set of analyses. A further set of analyses examined the relationships among the above tests, executive function and frontal lobe volumes; these analyses are discussed in a later section.

This study is an interesting attempt to integrate ideas of processing speed, Gf, Gc, working memory and executive function. However, it is unfortunate that, despite the strong correlation between working memory and Gf, the authors did not consider the use of further principal components analyses. Inclusion of Gc would also have been of considerable interest, as it would have helped to control for baseline differences in cognitive ability, which are known to be of considerable importance (Deary et al., 2000b). Because of these methodological issues it is difficult to interpret the results of the hierarchical multiple regression analyses used here. The study provides partial support to the processing speed hypothesis in that the age-related variance in Gf scores was almost eliminated when performance on the perceptual comparison speed was controlled for. This study also confirms that Gf is much more strongly related to age than Gc.

1.2.5 Conclusions

In young adulthood cognitive tests correlate to give rise to *g* and group factors. With ageing, the pattern of these correlations changes. Tests involving real-time problem solving, timing, and so on, tend to show decline and tests involving learned skills and knowledge (vocabulary, general knowledge, tend to increase). This phenomenon has been summarised by the *Gf/Gc* first proposed by Cattell and is related to the processing speed construct proposed by Salthouse. Whether or not there is a decline in specific cognitive functions in memory remains unclear.

In the literature neuropsychological and psychometric approaches can be identified, with neuropsychological models focusing on a variety of hypotheses linking decline in specific cognitive functions to atrophy of specific brain regions. Psychometric approaches are more concerned with the accurate measurement and classification of cognitive functions in relation to age, and in particular with examining the hypothesis that much age-related cognitive decline is due to a single pool of shared variance.

1.3 Ageing and the brain

The ageing brain can be studied using different levels and modes of analysis, from changes in neuronal DNA to changes in overall brain function, for example cognitive functioning. Because of the large literature on the ageing brain, this section will focus on macroscopic changes in the ageing brain together with a brief review of the processes thought to underlie macroscopic brain changes with ageing. Cognitive ageing, and ageing and glucocorticoids are covered in different sections.

As stated above, one of the key challenges in ageing research is in describing not just the universal changes which occur with ageing but also taking account of the varying degrees of pathological processes which become more common with ageing. For example, the evidence suggests that there is a degree of atrophy in the brain in all ageing individuals. There is a substantial amount of variance in this process and some of this can be attributed to disease processes such as Alzheimer's disease. However, most of these disease processes are not all-or-none, and post-mortem studies of the brains of apparently healthy older people commonly show features such as β -amyloid plaques which are present, albeit in greater quantities, in the brains of patients who had Alzheimer's disease.

1.3.1 Changes in the brain with ageing

In this section studies examining changes in the brain at the macroscopic level will be reviewed. The issue of changes in neuron number with ageing and in the context of pathology is also briefly considered.

In humans, brain weight is thought to decrease gradually from about the age of 60, even in the absence of Alzheimer's disease or other major pathologies. The brain volume to skull volume shows a similar decline, from 95% to about 80% in people in their ninth decade (Vinters, 2001). However, there is substantial variation, with some individuals showing little change, and others showing gross atrophy (Mueller et al.,

1998). Furthermore, there are different patterns of atrophy, with some regions showing more atrophy than others in some individuals (Laakso, 2002). The incidence of neurodegenerative diseases rises with age, and many of these are associated with brain atrophy. This means that it is difficult to know how much of the age-related change in brain volume is due to these illnesses, and how much to universal age-related change.

However, the literature does show that there might be distinct patterns of atrophy and that to some extent these are related to the severe cognitive impairments of the dementias. Using annual CT scans in a large prospective study, Jobst and co-workers (Jobst et al., 1994) found that patients with AD (confirmed with histology) showed dramatic declines in medial temporal lobe atrophy (15% reduction per year) versus healthy, age-matched controls (1.5% per year). They concluded that there is not an even distribution of decline and that rapid decline might reflect a qualitatively different process such as Alzheimer's disease. These findings have been replicated using MRI (Fox et al., 1996).

Cross-sectional studies have provided supportive evidence of heterogeneity in age-related atrophy of regional brain volumes. In one such study Laakso and co-workers examined hippocampal volumes in the context of Alzheimer's disease and age (Laakso et al., 1998). Subjects were 55 patients with probable AD (by standard criteria), 43 subjects with the National Institutes of Mental Health (NIMH) criteria for the clinical category of age-associated memory impairment, 42 cognitively normal healthy elderly controls and also 20 young healthy controls. Patients with AD had substantially and significantly smaller hippocampal volumes compared with all of the other groups. Hippocampal volume was not significantly different among the other groups. In particular, there was no difference between the young controls and the older controls or subjects with age-associated memory impairment. These and other studies (Laakso, 2002) suggest that hippocampal atrophy might be a marker of Alzheimer's disease; put another way, specific hippocampal atrophy is not a universal feature of ageing.

Other studies not explicitly examining patients with dementia have shown that although mean volume declines, not all people lose brain volume with age (Coffey et al., 1992) and that there might not be accelerated decline of brain volume loss with increasing age (Mueller et al., 1998). These studies strengthen the case for the existence of two or more subgroups of rates of decline rather than a general, uniform decline or an evenly-spread increased variability.

Good and co-workers (Good et al., 2001) used voxel-based morphometry, a technique allowing unbiased estimation of variation in all brain regions, to analyse the effects of age in 465 subjects selected for good health. Subjects were volunteers, and comprised 200 females aged 18 to 79 and 265 males aged 17 to 67; the age-range was skewed towards younger ages. Scans were all assessed as being “normal” by a neuroradiologist. Increased age was associated with relative declines in overall grey matter but not in overall white matter. Regional loss of grey matter was observed in several regions, including the superior parietal gyri, pre- and postcentral gyri, right cerebellum, anterior cingulate and left middle frontal gyrus. Somewhat surprisingly, areas of relative preservation of grey matter were the hippocampus, amygdala, and the entorhinal cortex as well as the thalamus. Regional loss of white matter was observed in the frontal regions, optic radiations, and the posterior limbs of the internal capsule. Areas of relative preservation were observed in the posterior frontal lobes, temporal lobes, and occipital lobes. The authors noted several limitations of the study: older subjects were relatively underrepresented and probably represented the healthy and high-functioning end of the spectrum, as older subjects with evidence of illness and also of any self-reported cognitive impairment were excluded. However, this study adds evidence to the proposal that brain ageing is heterogeneous; in particular it suggests that relatively greater hippocampal atrophy is not an inevitable consequence of ageing. This is a similar conclusion to that found in an earlier and relatively large study by Raz and co-workers (Raz et al., 1997). Using more conventional volumetric techniques in 148 healthy subjects aged 18 to 77, they found that the prefrontal grey matter was most affected by age, with smaller differences in the fusiform, inferior temporal and superior parietal cortices. There was only a slight effect of age on hippocampal volumes.

For many years it was almost universally believed that there was widespread neuronal loss with ageing (Coleman and Flood, 1987). However, this seems to have been due to artefacts in the only ways of counting neurons, which underestimated the numbers of neurons in 2-dimensional slides (West and Gundersen, 1990). West and co-workers used unbiased stereological techniques to examine patterns of neuronal loss in ageing in the brains of 45 subjects without Alzheimer's disease aged 13 to 101 and in 7 patients with Alzheimer's disease and 14 age-matched controls (West et al., 1994). They found that patients with AD showed loss of 68% of neurons in cornu ammonis area 1 of the hippocampus (CA1), 47% loss in the subiculum and 25% loss in the hilus of the dentate gyrus. In the 45 subjects without Alzheimer's disease, neuronal loss of 37% in the dentate hilus and 43% in the subiculum were found. There were no differences in CA1. No significant differences were found in CA2 or CA3 or in the dentate granule cell layer. Other studies have not found exactly the same pattern, with some showing that there is neuronal loss in CA1 with 'normal ageing' (Tang et al., 1997). Several other studies have confirmed the general principle that although there is some neuronal loss with ageing, this is not a large loss (in the order of 10%) and only seems to affect certain brain regions, such as parts of the hippocampus. This small neuronal loss does not fully account for the changes in brain volumes with ageing (Tang et al., 1997; Morrison and Hof, 1997; Peters et al., 1996; Rasmussen et al., 1996; Rapp and Gallagher, 1996).

Studies in rodents have yielded similar findings. For example, Rasmussen and co-workers (Rasmussen et al., 1996) examined hippocampal and subicular neuron number in relation to performance on memory tasks in aged rats. They first classified performance of 52 aged (24 to 24.5 months) rats on the Morris water maze in relation to 15 young controls (2.5 months). Aged rats performed significantly worse than young rats. However, as others have demonstrated (Issa et al., 1990; Yau et al., 1995), there was considerable variation in the performance of the aged rats, with a considerable overlap with the young rats. The aged rats were divided into groups of N=5 based on their watermaze performance with the best in one group and the worst in another. There was also a third group (N=5) randomly-selected from the young rats. Stereological counting of neuron numbers in the dentate granule layer, the

dentate hilus, the combined pyramidal cell layers of CA2 and CA3, the pyramidal cell layer of CA1 and the pyramidal cell layer of the subiculum was performed. They found that the impaired and unimpaired aged rats showed no differences in neuron numbers. The only significant difference found was between the CA1 neuron numbers in all 10 aged rats versus the 5 young rats in which the aged rats actually had a higher number of neurons.

Other than neuronal death, there are dozens of other variables which have been investigated as important factors in the ageing brain. Prominent amongst these are the deposition of β -amyloid plaques, which probably occurs in all ageing brains, increased neurofibrillary tangles with cells, loss of synapses and reduced numbers of dendrites (Anderton, 1997; Uylings and de Brabander, 2002), decreases in myelination (Nielsen and Peters, 2000), cerebral energy metabolism (Eberling et al., 1997; Yamauchi et al., 1996) and the integrity of the blood-brain barrier (Ueno et al., 2000).

1.3.2 Neuroimaging and cognitive ageing

Various neuroimaging techniques are being used to examine relationships between the brain and cognitive function in health and disease across the age range. In this section relationships between neuroimaging techniques generating data on brain volumes and metabolite levels (magnetic resonance spectroscopy) and cognitive function will be reviewed.

Brain volumes

In young, healthy adults, most studies have found a positive correlation between brain size (as measured by various neuroimaging techniques) and cognitive ability (Deary et al., 2000a). Vernon and co-workers reviewed the literature in 2000 and found that for the 11 published studies (total $N=432$) the N -weighted mean

correlation between IQ and brain size was 0.38 (Vernon et al., 2000). However, in ageing research, the majority of the literature on cognitive function, brain volumes and ageing is concerned with the dementias, examining hippocampal volumes and performance on tests of memory in the context of the prediction of Alzheimer's disease (deLeon et al., 1997). There are few studies which have examined relationships between comprehensive cognitive testing and brain volumes beyond the hippocampus in older individuals.

Swan and co-workers examined relationships in 385 males with a mean age of 72 among a battery of cognitive tests, including the mini-mental status examination (MMSE), visual and verbal memory, verbal fluency, vocabulary, colour-word interference (similar to the Stroop test), the Trail-Making Test and the Digit-Symbol Substitution Test (DSST) in relation to structural neuroimaging data and risk factors for cerebrovascular disease (Swan et al., 2000). Some of the cognitive tests had been administered to all of the subjects 10 years previously. The investigators divided the subjects into those with 'atrophy' (n=101) versus 'non-atrophy' (n=284). Atrophy was defined as, "... a measurement of total brain volume less than the median and white matter hyperintensities volume more than the median for this sample." No correlational analyses were done; all inferential statistics were done with logistic regression after adjustment for age. Subjects with scores on the MMSE of 23 or less were excluded. They found that the groups differed in performance on the Trail Making Test, colour-word interference, verbal fluency and 10-year change in verbal fluency and the DSST. There were no differences in visual or verbal memory. This study does provide some evidence for a link between brain atrophy and age-related cognitive decline but the dichotomisation of the continuous variable of total brain volume is not the appropriate method for analysing this relationship (MacCallum et al., 2002). The authors use the term 'subclinical brain atrophy' to describe the group with the lower brain volumes but there is no agreed definition of clinical atrophy.

Tisserand and co-workers (Tisserand et al., 2000) examined relationships among cognitive function and brain volumes in 61 healthy men and women aged 21 to 81 (mean 55.7, standard deviation 16.1). Cognitive tests were only of immediate and

delayed word-list recall, a test of perceptual processing speed (the 'Memory Scanning Test'¹) and two versions of the Stroop test. Neuroimaging measures were intracranial volume, total brain volume, hippocampus, parahippocampal gyrus, mamillary bodies and volume of the third ventricle. All brain volumes were controlled for intracranial volume. They found significant age-related declines in all brain regions apart from the mamillary bodies. No measured region showed more decline than any other. There was also a significant rise in the volume of the third ventricle. Relationships among cognitive test scores, brain volumes and age were examined with hierarchical regression models. Without the inclusion of age, all measured brain volumes other than the mamillary bodies were significantly associated with the Memory Scanning Test and one of the Stroop test scores. Immediate word list learning was not associated with hippocampal volumes but was associated with total brain volumes, as was delayed word list learning. Controlling for age eliminated all of the associations between cognitive tests and brain volume other than the more difficult version of the Memory Scanning Test (MST 3) and total brain volume. This study, though it used a small number of cognitive tests and did not control for baseline cognitive ability, does add to the idea that in the absence of overt pathology, relationships among cognitive ability and brain volumes are general rather than involving specific relationships between specific brain regions and specific cognitive domains.

As stated above, there are many studies which have examined the relationship between hippocampal volumes and declarative memory, mainly in older populations with cognitive impairment of varying degrees of severity (Scheltens et al., 2002; Zaudig, 2002). These studies often show the predicted negative relationships, but

¹ this task was described as follows (p. 571): "The Memory Scanning Test is a paper and pencil cancellation test that consists of 12 rows of 12 letters and numbers, randomly interspersed with a target symbol or letter that subjects have to cross out. The cognitive load can be enhanced by increasing the number of targets that need to be memorized. In the present study, two targets were used: one with one target symbol (MST%) and one with three letters (MST3), and the time needed for completion was recorded."

alternative explanations for the relationships observed, such as a correlation between overall brain size and general cognitive ability, are often not tested.

Magnetic resonance spectroscopy

Proton magnetic resonance spectroscopy (H1-MRS) can measure levels of certain metabolites in the brain in vivo. Commonly identified metabolites are N-acetylaspartate (NAA), choline (Cho), and total creatine (Cr). NAA is considered to be neuron-specific (Simmons et al., 1991) and is considered to be a marker of normal functioning neurons. Choline levels reflect the quantities of free choline. Since choline-containing compounds are contained in phospholipid membranes, increased choline may reflect increased membrane synthesis (eg. in gliosis) or breakdown (eg. in necrosis) (Pettegrew et al., 1997). Creatine levels are thought to reflect the combined quantity of phosphorylcreatine and creatine, involved in energy metabolism in neurons and glial cells (Pettegrew et al., 1995; Wyss and Kaddurah-Daouk, 2000).

H1-MRS is increasingly used in the study of neurodegenerative diseases. For example, decreased levels of NAA have been found in pathological states in which there is known to be neuronal loss, such as Alzheimer's disease (Pfefferbaum et al., 1999a; Schuff et al., 1998), epilepsy (Breiter et al., 1994; Aasly et al., 1999), Huntington's disease (Sanchez-Pernaute et al., 1999) and schizophrenia (Deicken et al., 1999).

Magnetic resonance spectroscopy and cognitive ageing

The majority of published studies have examined metabolite levels, usually in the form of metabolite ratios, in relation to Alzheimer's disease. In these studies there a mixed picture of results, though most suggest a relative reduction in NAA in comparison to healthy brains (Firbank et al., 2002; Pfefferbaum et al., 1999a).

There are very few studies of metabolite changes in relation to changes in cognitive function with ageing. In a study of otherwise healthy people aged over 70, Catani and co-workers reported higher myo-inositol/creatine ratios in 11 persons with mild cognitive impairment versus 11 healthy controls (Catani et al., 2001). Pfefferbaum and co-workers reported a positive correlation between NAA levels and a face recognition task in 13 healthy elderly subjects (Pfefferbaum et al., 1999b). Even fewer studies have been published in young, healthy adults. One group reported a positive association between absolute NAA concentrations and performance on cognitive tests in 40 subjects (Jung et al., 1999). Overall, the literature to date does not allow firm conclusions to be drawn concerning changes in brain metabolites with ageing.

1.4 Glucocorticoids

1.4.1 Glucocorticoid physiology

The main function of the glucocorticoid hormones (cortisol in humans, corticosterone in rodents) is to enhance the organism's response to physical or psychological stress (Seckl and Olsson, 1995). The presence of glucocorticoids is also crucial in maintaining the normal functioning of multiple physiological systems. Deficiency of glucocorticoids, such as in Addison's disease or in animal experiments through adrenalectomy, causes widespread adverse effects under basal conditions and can lead to death under conditions of stress (Kaplan, 2000).

The response to stress is complex and involves multiple components of the endocrine and nervous systems. In the first few seconds and minutes the main physiological actions are an increase in the supply of fuel to muscle, increased blood pressure, inhibition of reproductive physiology, decreased appetite, and increased alertness (Sapolsky et al., 2000). Many of these very early responses are mediated by the nervous system and by the catecholamines, secreted from the adrenal medulla after stimulation from the sympathetic nervous system. Glucocorticoids are secreted immediately after stress but in the main do not act for at least 20 to 30 minutes because most of their actions are genomic.

The roles of glucocorticoids appear to involve the facilitation of the initial response, but also the restoration of the basal state. Glucocorticoids also influence the organism's preparation for later stress (Sapolsky et al., 2000). Central to the glucocorticoid response to stress is mobilisation of glucose, gluconeogenesis and inhibition of energy storage (Kaplan, 2000). There are also multiple adaptive effects on the brain. Although stress causes an immediate increase in glucose uptake by the brain, probably mediated by catecholamines and the sympathetic nervous system, stress-levels of glucocorticoids inhibit cerebral glucose uptake from about one hour after the episode of stress (Kadekar et al., 1988; Doyle et al., 1993). Glucocorticoids

also influence the formation of memories and influence other cognitive functions; this is discussed below.

Glucocorticoids are released under the influence of adrenocorticotrophic hormone (ACTH), a peptide, which is released from the pituitary gland when stimulated by corticotrophin-releasing hormone (CRH) from the median eminence of the hypothalamus. ACTH binds to a membrane receptor, activates adenylyl cyclase and activating protein kinases which stimulate the manufacture of cortisol from cholesterol. Cortisol levels increase within a few minutes of stimulation with ACTH to between two and five times basal levels in about 30 minutes. Elevations in cortisol occur as part of the normal circadian rhythm: in humans, cortisol levels are at their lowest between 12 midnight and 0400, rising to a peak at around 0800 and showing a slow decline over the course of the day. Elevations also occur with a stressful episode; glucocorticoids can increase to levels higher than the peak levels observed in the normal circadian rhythm (Roy et al., 2001b).

In the circulation glucocorticoids are mainly bound to a specific glucocorticoid-binding α 2-globulin, corticosterone-binding globulin (CBG). CBG is saturated at a glucocorticoid level of approximately 700nmol/l; the remainder is bound to albumin or is unbound. The unbound fraction is about 5%. The plasma half-life of cortisol is between 70 and 90 minutes. It is either metabolised to cortisone, or excreted in the urine unchanged or as 5b-tetrahydrocortisol (Seckl and Walker, 2001).

Receptors

Glucocorticoids are steroid hormones and enter cells by passive diffusion. Glucocorticoids bind to two types of receptors: mineralocorticoid (MR or type I) and glucocorticoid (GR or type II) receptors (de Kloet et al., 1998). GR and MR are situated in the cytoplasm and are complexed with other proteins. The hormone-receptor complex translocates to the nucleus and binds to specific regulatory DNA sequences called glucocorticoid response elements, influencing the transcription of

hundreds of different genes (Vreugdenhil et al., 2001). Glucocorticoid receptor monomers may also act indirectly by interacting with transcription factors and influencing transcription rates of multiple genes (Reichardt et al., 1998).

MR have about 10 times the affinity of GR to physiological glucocorticoids but are thought to have a lower capacity than GR (de Kloet et al., 1998). In the brain MR are almost 100% occupied by glucocorticoids, their main ligand in the brain, under basal conditions. By contrast, GR are of lower affinity than MR and in the brain become occupied only when glucocorticoid levels are at stress levels (Spencer et al., 1990). De Kloet and others have proposed that this allows the brain to distinguish between stress and circadian levels of glucocorticoid (de Kloet et al., 1998).

In rodents, MR are clearly present in the hippocampus, the amygdala, the basal ganglia, the hypothalamus, the cerebellum, the eye and in the distal nephron (Sutanto et al., 1988; de Kloet et al., 1998; Agarwal et al., 1993; Mirshahi et al., 1997). One recent report indicates that MR are also expressed in the neocortex in non-human primates (Patel et al., 2000). GR are present throughout the brain and in numerous tissues throughout the body (Seckl and Olsson, 1995). Inter-species densities of GR vary, with some reports showing higher GR expression in the prefrontal cortex and other parts of the neocortex in non-human primates as compared with rodents (Sanchez et al., 2000; Patel et al., 2000).

Feedback regulation of the hypothalamo-pituitary-adrenal axis

There is a complex set of feedback loops which regulate cortisol levels. Glucocorticoids exert negative feedback on the adrenal cortex itself, the pituitary gland and the hypothalamus. Several cortical regions are also involved in feedback regulation including the hippocampus (Seckl and Olsson, 1995) and the medial prefrontal cortex (Diorio et al., 1993).

The hippocampus is thought to have a tonic inhibitory effect on CRH production by the hypothalamus. In rats, lesions to the hippocampus result in elevated

corticosterone levels and slower recovery of hormone levels following stress (Sapolsky et al., 1984; Wilson et al., 1980; Feldman and Conforti, 1980). Animals without hippocampi show increased CRH levels in the paraventricular nucleus (Herman et al., 1989). Studies have demonstrated that both GR and MR appear to have a role in feedback regulation. Administration of an MR antagonist via an intraventricular catheter to rats causes elevations in basal levels of corticosterone and ACTH, and also larger corticosterone responses to novelty (Ratka et al., 1989; Oitzl et al., 1995).

Similar findings have been reported in non-human primates (Sapolsky et al., 1991) and in humans, where elevations of cortisol levels are observed following blockade of MR by spironolactone (Young et al., 1998; Heuser et al., 2000) or canrenonate (Arvat et al., 2001).

However, although the hippocampus undoubtedly has an important role in hypothalamo-pituitary-adrenal (HPA) axis feedback regulation the main site is thought to be the pituitary (de Kloet et al., 1998). It is also important to note that some studies have suggested that the impaired HPA axis feedback regulation observed after hippocampal lesions recovers after several weeks or months (Sapolsky et al., 1991), though the mechanism for this is unknown.

Glucocorticoid sensitivity

The strength of response to circulating glucocorticoids depends on multiple factors. The first group of factors concerns local modulation of glucocorticoid levels. Although glucocorticoids enter cells by passive diffusion, there are several mechanisms which modify the quantity of hormone actually reaching MR and/or GR.

As stated above, most glucocorticoid is carried bound to CBG; reductions in CBG lead to higher concentrations of active (free) hormone. Recent studies have demonstrated that CBG levels may be locally altered by inflammation, for example

by neutrophil elastase, which cleaves CBG so that local glucocorticoid levels are enhanced (Hammond, 1995).

Another important mechanism of local tissue modulation of hormone levels is via enzymes called 11 β -hydroxysteroid dehydrogenases (11 β -HSDs). In the distal nephron the main ligand for MR is aldosterone. However, glucocorticoids typically have much higher concentrations than aldosterone, and given that MR has a high affinity for both glucocorticoids and aldosterone *in vitro*, how does aldosterone have preferential access to these receptors *in vivo*? This paradox was explained by the discovery of 11 β -HSDs, which are co-localised with MR in the distal nephron and in that site inactivate glucocorticoids before access to MR occurs. Thus, aldosterone, present in lower concentrations, occupies MR (Edwards et al., 1988; Funder et al., 1988). The syndrome of apparent mineralocorticoid excess, in which there is sodium retention, hypokalaemia and hypertension, was found to be due to congenital absence of 11 β -HSDs, allowing mineralocorticoid receptors to be occupied by active glucocorticoids. Inhibition of 11 β -HSDs by substances such as carbenoxolone also causes this effect.

There are two isozymes of 11 β -HSD: type 1 and type 2. 11 β -HSD type 1 (11 β -HSD1) is widely expressed, in liver, lung, adipose tissue, vasculature, ovary and brain (Stewart and Krozowski, 1999; Monder and White, 1993). To date, the bulk of the evidence suggests that 11 β -HSD1 acts as a reductase *in vivo*, that is, it regenerates glucocorticoids from their inert metabolites (Rajan et al., 1996; Kotelevtsev et al., 1997; Seckl and Walker, 2001). By contrast, 11 β -HSD type 2 (11 β -HSD2) is present only in the distal nephron, the colon, salivary glands, the placenta and a few regions in the central nervous system which are involved in central blood pressure and salt and water regulation (Seckl, 1997). 11 β -HSD type 2 is thought to function as a dehydrogenase, that is, it deactivates active glucocorticoids.

A further type of local modulation applies to synthetic analogues of glucocorticoids, such as dexamethasone. Many synthetic compounds are denied access to the brain via the *mdr1a* P-glycoprotein (Schinkel et al., 1995). This is expressed in the endothelial cells of the blood-brain barrier (Cordoncardo et al., 1989). Mice lacking

the *mdr1a* P-glycoprotein show greater accumulation in the brain of radiolabelled dexamethasone than wild-types (Meijer et al., 1998). Therefore, in mice the brain appears to be resistant to penetration of dexamethasone, though high doses can overwhelm this system. These data are relevant to the site of action of exogenous steroids.

1.4.2 Glucocorticoids, cognitive function and ageing

The hypothesis that elevated levels of glucocorticoids are causally associated with ageing-related cognitive decrements was proposed several decades ago and was developed by Sapolsky in the glucocorticoid cascade hypothesis (Sapolsky et al., 1986). This hypothesis states that prolonged periods of high glucocorticoid levels lead to a down-regulation of GR in the hippocampus. If this process continues feedback regulation of the HPA axis is attenuated because the hippocampus exerts an inhibitory influence on glucocorticoid levels. This leads to a further increase in glucocorticoid levels and eventually to damage and even death of hippocampal neurons, causing impairments in delayed memory.

In this section the literature on ageing-related changes in the functioning of the HPA axis, in particular glucocorticoid levels, is reviewed. Then literature on the effects of elevated glucocorticoids on the brain and cognitive function, in health and in ageing, is reviewed.

Glucocorticoids and ageing: observational studies

The impact of ageing on the HPA axis can be measured in several ways. In terms of basal glucocorticoid secretion, levels can be measured in saliva, blood or urine. Urine sampling allows glucocorticoid secretion over 24 hour periods to be measured, whereas saliva and blood sampling tend to be done as a one-off or a small number of times during the day. As well as observational measurements, measurements of glucocorticoid levels can be measured when the organism is subjected to stress or challenge. For example, cortisol levels in humans can be measured under situations of social stress such as public speaking (Roy et al., 2001a). Dexamethasone, a synthetic glucocorticoid, acts on various feedback sites and causes suppression of glucocorticoid secretion. Individuals differ in their sensitivity to this test. Post-

mortem studies can be used to examine levels of MR and GR in the brain and also of parts of the brain thought to be involved in HPA axis regulation and/or vulnerable to the effects of elevated glucocorticoids.

There is uncertainty over whether overall levels of glucocorticoids increase with ageing (Seeman and Robbins, 1994; Sapolsky, 1991; Seckl and Olsson, 1995). Studies have tended to show either no changes, or slight increases (Barton et al., 1993). Some of these differences can be put down to differences in methods of measuring glucocorticoid levels, with studies using morning or evening plasma levels, 24 hour urine output, etc. (Van Kampen and Fuchs, 1998). However, what is in little doubt is that the average output of glucocorticoids shows a wider range, with some individuals maintaining the same overall output and others showing decreases or increases. For example, Lupien and co-workers measured cortisol levels in 51 volunteers yearly for three to six years (Lupien et al., 1996). The subjects were aged 60 to 90, with a mean age of 73 years. Cortisol levels were sampled in a highly detailed manner, using samples taken every hour over a 24-hour period through a forearm catheter. They found that cortisol levels showed a spread of change, and divided the subjects into three groups: those with rising levels, those with stable levels, and those with decreasing levels. Other studies have confirmed considerable variance in cortisol secretion in older population (Seeman et al., 1997).

There are also differences in the dynamics of glucocorticoid secretion. With respect to circadian rhythms, some studies have reported higher morning and/or evening cortisol levels, whereas some others have reported no such difference (Ferrari et al., 1995; Vermeulen et al., 1982; Kern et al., 1996). However, as with overall output of glucocorticoids with ageing, there is no clear pattern which occurs with ageing in humans.

Most of the research on the functioning of the HPA axis under conditions of stress has been done with animals, using a variety of stressors, including restraint, cold, and open field testing. Most of these studies have found that the consistent differences have occurred in the period when hormone levels are returning to their basal levels, in that older animals show a slower recovery (Seeman and Robbins, 1994). For

example, in one study stress caused by injections and blood sampling caused a similar peak in corticosterone levels in young and old animals, but levels in older animals took longer to return to basal levels (Vaneeckelen et al., 1995). However, not all aged animals show this slower recovery (Issa et al., 1990; Bizon et al., 2001), paralleling the findings with overall glucocorticoid output. There are no good quality published studies examining the recovery of cortisol levels following stress in older humans.

There are several published studies examining the effects of age on the extent to which dexamethasone suppresses HPA axis activity. Studies differ in many ways, including the numbers of subjects, age groups, exclusion criteria and importantly, the dose of dexamethasone used. Studies also often report the findings in terms of the percentage of subjects showing suppression versus non-suppression dichotomy rather than reporting the mean levels. Studies have tended to show that older subjects show higher levels of cortisol post-dexamethasone than young controls (Weiner, 1989; Rosenbaum et al., 1984). This effect is more clearly seen when smaller doses of dexamethasone are used (Branconnier et al., 1984). However, the overall pattern is that in healthy older people there is a spread of response to dexamethasone whereas in the absence of illness in young people, virtually all completely suppress output of cortisol in response to dexamethasone. Similar effects have been found in rodents.

In conclusion, it is uncertain whether some older humans or animals develop a higher overall output of glucocorticoids. Similarly, the evidence for clear age-related alterations in the circadian rhythm is scant. However, there is abundant evidence that in a proportion of older animals and humans there is an impairment of negative feedback at various levels of the HPA axis, resulting in a slower recovery of glucocorticoid hormones to resting levels. Reviewing evidence from animal and human studies Seeman and Robbins (Seeman and Robbins, 1994) concluded that, "... aging *per se* is probably not associated with changes in HPA function but rather than there are *differential patterns of HPA aging ...*" (p.234).

1.4.3 Chronic exposure to high levels of glucocorticoids and cognitive function

Glucocorticoid hormones are essential for normal cognitive functioning (de Kloet et al., 1999). Both MR and GR have been shown to play a role. The key findings are that the involvement of glucocorticoids in learning varies according to the situational and temporal context. If glucocorticoid levels are too low, or too high, or the functioning of MR or GR is affected, learning is attenuated. For example, Yau and co-workers have demonstrated that MR activation is important in maintaining memory in healthy rats. In a controlled study they administered intraventricular spironolactone, a specific MR antagonist, or vehicle for 12 days and found that this impaired learning in the Morris watermaze (Yau et al., 1999). Similar studies have shown that blockade of GR also impairs learning.

In the next sections, the effects of chronically high levels of glucocorticoids on cognitive function and the brain are reviewed.

Animal studies

Observational animal studies

Landfield and co-workers were the first to report quantitative data linking ageing of the HPA axis with brain ageing. This group had previously reported an increase in reactive astrocytes in the hippocampus and other brain structures with ageing in Fischer rats, and went on to examine the hypothesis that plasma levels of corticosterone and adrenal weights were related to the extent of these changes (Landfield et al., 1978). They compared three groups of 9 rats of three age groups: 4, 13 and 25 months old. They found progressively higher densities of reactive astrocytes and adrenal weights with increasing age. Mean corticosterone levels also

increased with age, though the authors noted that this was because of two outliers in the aged group; median levels actually showed a slight decrease compared with the middle-aged group. In the conclusions section of the paper they acknowledged that the existence of a correlation, whilst intriguing, did not prove any causative role for elevated corticosterone levels in brain ageing, or vice versa.

Probably the most important study in this area was provided by Issa and co-workers, who screened 58 aged rats (23-27 months) for impairments in spatial memory with the Morris water maze (Issa et al., 1990). Animals with performance greater than two standard deviations worse than young controls were classified as impaired (28%) and animals with performance within half of one standard deviation of the young controls were classified as being unimpaired. They found that compared to the unimpaired aged animals and young controls the impaired group had higher basal corticosterone levels. They also found that under conditions of restraint stress the impaired animals showed a relatively slower recovery of corticosterone levels to basal levels despite sharing the same peak levels of corticosterone with the unimpaired animals and young controls. A recent study reported the same pattern of results: older animals differed in spatial ability and this difference correlated with the speed of recovery of corticosterone to basal levels (Bizon et al., 2001).

However, other studies have failed to show relationships between impairments in cognitive function and indices of HPA function. Yau and co-workers found no relationship between corticosterone levels and performance on the Morris water maze in aged Wistar rats, though the normal age-related decrement in performance was observed (Yau et al., 1994). They also found that there were no changes in the expression of GR and MR in the hippocampus in aged versus young rats.

Interventional animal studies

Following on from their observational study correlating corticosterone levels to ageing-related changes in the hippocampus, Landfield's group tested the hypothesis

that long-term reductions in corticosterone levels are associated with a reduction in ageing-related decrements in spatial memory and changes in the hippocampus (Landfield, 1981). There were several experimental groups, which are summarised in Table 1.3. Interventions were either drug (ORG 2766, an ACTH analogue; pentylenetetrazole (PTZ), a 'neural stimulant'), or adrenalectomy with glucocorticoid replacement. Outcome variables were performance on a delayed-memory maze task² and several hippocampal morphological measures of variables which had previously been shown to show change with ageing, including neuronal density, changes in neuronal nuclear morphology. A "brain aging index" was also derived from a combination of the morphological variables.

The main findings were that the young controls and the drug treatment groups showed significantly better performance than the aged controls and the adrenalectomised group on the maze task. There was no significant difference between the aged controls and the adrenalectomised group on this task, though a trend is evident from examining the bar charts. However, there were differences in several indices of morphological change and in the 'brain aging index'.

² the task was described as follows (p. 584, note 13): "Animals were trained in a cross-shaped maze (three arms plus one start box) to choose one arm (left) to avoid or escape footshock: after 1 week, they were then reversed and trained to the right arm. A footshock trial consisted of placing a rat in the start box and after 5 seconds delivering shock (0.35 mA) to the grids of the start alley and to the two incorrect maze arms until a correct choice was made. On nonfootshock trials rats were placed in the maze until a choice was made, or until 2 minutes had elapsed, but no shock was given. At the beginning of initial training, three acquisition footshock trials were given, and animals were then tested for retention performance by giving them one nonfootshock trial, and one footshock trial on each of days 1, 4, 5 and 6 after the acquisition session. Two (reversal) acquisition footshock trials were then given for the reversal phase, and animals were subsequently tested in the same manner and sequence as after the initial phase acquisition trials. Mean latencies (time from start to correct choice) on footshock trials were subjected to analyses of variance. Acquisition footshock trials were not included in this analysis since the study was aimed at assessing retention performance (although reversal data are also significant if acquisition trials are included)."

Table 1.3: Experimental groups in Landfield's 1981 experiment

Group	Initial N	Final N	Age at onset and end (months)	Intervention
(1) adrenalectomy and glucocorticoid replacement	19	11	16,27	Adrenalectomised at 18 months and maintained on low-dose glucocorticoid
(2) ORG 2766	12	10	16,27	Injection of ORG 2766 once per day, five times per week, for 8.5 months.
(3) PTZ	12	10	16,27	Injection of PTZ once per day, five times per week, for 8.5 months.
(4) Vehicle or non-vehicle controls	33	20	16,27	Injection of vehicle once per day, five times per week, for 8.5 months, or no treatment (authors analysed these groups together)
(5) Young-mature control	11	11	0,8	No treatment

Despite the considerable interest in this study (it has been cited hundreds of times) there have been no attempted replications published. Thus there is little strong evidence that an excess of *endogenous* glucocorticoids is causally related to changes in the brain with ageing and parallel decrements in cognitive function.

However, there are several studies which have examined the effects on cognitive function and the brain of long-term administration of exogenous glucocorticoids. Virtually all of the studies have focused on the hippocampus; few other brain regions have been studied in this context. Most studies have shown that chronic exposure to

glucocorticoids does result in changes in the hippocampus, for example loss of third-order dendrites (Seckl and Olsson, 1995), but not perhaps the extensive neuronal loss as was originally suggested (Sapolsky et al., 1986). Studies suggest that it is the CA3 field of the hippocampus which appears to be especially vulnerable to glucocorticoid exposure. For example, Woolley and co-workers showed that in rats three weeks of treatment with high dose corticosterone causes a reversible regression of apical dendrites of neurons in the CA3 field of the hippocampus (Woolley et al., 1990). Another study found that in primates long-term (one year) implantation of cortisol pellets in the hippocampus caused neuronal shrinkage and dendritic atrophy in the hippocampal subfields of CA2/3 (Sapolsky et al., 1990).

Since the development of unbiased stereological counting techniques, very few studies have been published revisiting the hypothesis that chronic exposure to high levels of glucocorticoids actually cause neuronal death, particularly in the hippocampus. Sousa and co-workers compared several different regimes of administration of corticosterone in rats (Sousa et al., 1998). Rats were treated in the neonatal period for 30 days before histological analysis. In the neonatal category the experimental groups were, control, vehicle-treated, or corticosterone-treated (subcutaneous injections at 10mg/kg on days 0-10, 20mg/kg on days 11-20, and 30mg/kg on days 21-30). In the adult group the experimental groups were controls, given no corticosterone and also no injections of vehicle at any stage, a 'recovery' group, which received no corticosterone beyond the 30 day neonatal treatment period, and three corticosterone-treated groups. All of the corticosterone groups were treated as per the neonatal group, then given either (i) 40mg/kg for the remainder of the 180 day period, or (ii) 40mg/kg from days 90-180, or (iii) 40mg/kg from days 150 to 180. Corticosterone-treated rats showed lower brain weights than vehicle-treated groups. Corticosterone treatment also caused a reduction in the number of dentate granule and CA3 pyramidal cells, however this effect was only seen in rats treated in the neonatal period. That is, adult animals treated with corticosterone did not show neuronal loss compared with adult animals which received no treatment. However, the adult-treated animals did show loss of volume in the dentate gyrus and in CA3. The recovery group also showed greater volumes of the dentate gyrus in

comparison to the treated groups. Overall, there was a significant effect of corticosterone treatment on overall hippocampal volume for both the 30-day old and the 180-old groups. This study does add weight to the hypothesis that prolonged exposure to high levels of glucocorticoids causes hippocampal atrophy and that this seems to affect CA3 more than CA1. A potential confounder in this study, however, is that the aged control group received no injections and therefore one cannot rule out the general effects of stress caused by injection rather than the direct effects of high corticosterone levels.

Leverenz and co-workers (Leverenz et al., 1999) conducted a placebo-controlled prospective study of the effects of high-dose cortisol supplementation on 16 macaque monkeys aged between 18 and 29. Monkeys were given cortisol or placebo for one year. Cortisol exposure made no difference to total or subfield hippocampal volumes and also made no difference to neuronal numbers. The authors did not report on any dendritic changes.

The relationship between decrements in cognitive function and exposure to high levels of exogenous glucocorticoids is less clear. For example, Bardgett and co-workers tested the effects of chronic corticosterone administration on performance on a test of spatial memory, the Platform Maze Task (Bardgett et al., 1996). This task had previously been shown to be sensitive to hippocampal dysfunction (Poucet et al., 1991). Two groups of 13 male Long-Evans rats aged 80-100 days old were given eight weeks of either daily injections of corticosterone (10mg/kg) or vehicle. This group had previously published evidence that this regime produced stress levels but not supraphysiological plasma levels of corticosterone. No differences between the groups were found on any of the task outcome variables. No analysis of brain was included in this report.

Another group of studies has looked at the effects of antidepressants on corticosterone levels and age-related cognitive decrements in animals, based on the observations that (1) in some studies hippocampal MR and GR are reduced and (2) antidepressants have been shown to increase expression of hippocampal MR and GR

in young rats, thus enhancing feedback sensitivity of the HPA axis (Seckl and Olsson, 1995; Yau and Seckl, 2001).

Yau and co-workers administered long-term amitriptyline to young and aged Wistar rats and examined effects on Morris water maze performance and also on expression of hippocampal MR and GR (Yau et al., 1995). They found that amitriptyline treatment only affected young rats, in terms of increased performance and higher expression of hippocampal MR, but not hippocampal GR. Compared with the young rats, aged rats did show worse performance on the watermaze and had higher basal corticosterone levels. Interestingly, in the aged rats, hippocampal GR expression was positively associated with watermaze performance and negatively associated with corticosterone levels.

A similar study was conducted with a different breed of rats, the Lister-hooded, as the Wistar rats in the original study had shown difficulties in learning the watermaze task across the age range (Yau et al., 2002). Rats were treated from 16 months with amitriptyline or vehicle and at 24 months. Corticosterone levels were measured at 22 months. Performance on the watermaze and levels of anxiety as measured by the elevated-plus maze were measured at 24 months. Amitriptyline-treated rats showed significantly better performance than vehicle-treated rats, with virtually all showing comparable performance to the young control group. The mean performance of aged rats treated with placebo was lower in comparison to a young control group, but there was considerable overlap between the aged and young rats, with around one third of the aged rats showing no impairment, as had been previously observed (Issa et al., 1990). Amitriptyline-treated rats also showed lower corticosterone levels and lower levels of anxiety.

Further and compelling evidence that elevated glucocorticoids might affect vulnerable regions of the brain has emerged with the development of a mouse with the gene for 11 β -HSD1 knocked out. 11 β -HSD1 is thought to function predominantly as a reductase *in vivo*, regenerating active glucocorticoids from their inactive metabolites (Rajan et al., 1996). Thus it was hypothesised that mice lacking the 11 β -HSD1 gene would show less age-related cognitive decline because of

reduced exposure of neurons containing 11 β -HSD1 to active glucocorticoids – ‘intraneuronal’ glucocorticoids as opposed to plasma levels. Yau and co-workers compared watermaze performance and corticosterone levels in aged wild-type and 11 β -HSD1 knockout mice. The knockout mice showed significantly better performance on the watermaze despite having higher mean corticosterone levels (possibly because of reduced feedback to the HPA axis). Wild-type mice showed the predicted higher variation in performance and corticosterone levels seen with ageing rats and corticosterone levels were negatively correlated with watermaze performance.

Human studies

Cushing’s syndrome

Cushing’s syndrome is characterised by chronic excess of glucocorticoids, largely caused by ACTH-secreting tumours in the pituitary gland and elsewhere, or by chronic excess of exogenous steroids (Williams and Dluhy, 2001). Cushing’s syndrome is of considerable interest as there is a relatively pure increase in cortisol levels. This disorder thus serves as a model for understanding the effects of cortisol hypersecretion on multiple systems. This is in contrast to other conditions in which hypercortisolaemia is seen, such as depressive illness, in which there are many other hormonal and neurotransmitter abnormalities.

Patients with Cushing’s syndrome commonly have abnormalities in multiple systems: centripetal obesity, increased weight, tiredness, hypertension, hirsutism, amenorrhoea, purple cutaneous striae, depression and other psychiatric changes, cognitive impairment, osteoporosis, hyperglycaemia with diabetes mellitus in some patients, bruising, proximal myopathy and fluid retention. There is a higher excretion

of urinary free cortisol and plasma cortisol levels show little or no suppression with dexamethasone.

One of the first reports of links between Cushing's syndrome and changes in the brain was provided by Momose and co-workers (Momose et al., 1971).

Pneumoencephalography was used to study the 31 patients with Cushing's syndrome. They found that 90% of cases met criteria for atrophy of the cerebrum and the cerebellum. The same techniques were applied to 64 patients with acromegaly; 30% were found to have atrophy. The authors noted that the appearances of atrophy were similar to those observed in many patients with old age.

Other published studies have replicated this finding. Starkman and co-workers demonstrated that patients with Cushing's syndrome showed atrophy of the hippocampus in association with deficits in verbal memory (Starkman et al., 1992). More recently the same group reported that in 22 patients with Cushing's disease, treatment caused an increase in the volume of the hippocampus; the 'control' region of the head of the caudate nucleus showed no such increase post-treatment (Starkman et al., 1999). Unfortunately no other brain regions were studied.

There are several studies examining the impact of Cushing's syndrome on cognitive function and mood. Whelan and co-workers administered a large battery of cognitive tests, including the full WAIS and tests of delayed verbal memory, to 35 patients with Cushing's syndrome before treatment (Whelan et al., 1980). They compared patients' scores on this battery with the norms provided for the battery of tests. Patients were classified into four groups on the basis of their performance, with Group I being normal or equivocal performance and Group IV being frequent and marked signs of neuropsychological deficits. 22 of the 35 patients were classified as being in the clearly impaired groups. The deficits were present in virtually all tests: "... no function was spared; all tests of higher (cognitive) and lower (sensory and motor) functions showed marked defects in at least one patient." (p.755). No group of tests appeared to be affected more than the others. However, this study was weakened by the lack of a control group; the use of norms fails to take account of pre-morbid ability. Starkman and co-workers did a similar study in 48 untreated

Cushing's syndrome patients and 38 healthy controls (Starkman et al., 2001). They used standard test batteries: the WAIS-R and the Wechsler Memory Scale-Revised, and assessed mood with two standard scales. Significant differences between patients and controls were found on *all* of the cognitive tests, though the differences were not large. For example, mean scores on delayed paragraph recall (Logical Memory II) were 8.4 (SD 3.3) for the control group and 6.2 (SD 2.5) for the patient group. However, no measures of premorbid or prior IQ such as the NART were used and therefore differences cannot with certainty be attributed to the effects of chronic exposure to higher levels of cortisol. However, the general pattern of results is similar to Whelan's study. Forget and co-workers (Forget et al., 2000) studied 19 patients with Cushing's syndrome in relation to controls matched for age, sex, occupation. Subjects completed a large battery of cognitive tests, including standard tests of memory and intelligence. They found widespread deficits in the patients' performance. These small studies suggest that (1) chronic exposure to high levels of cortisol is associated with cognitive decrements and that (2) these cognitive decrements are general, that is, no particular cognitive domain is affected more than any other.

Human observational studies

There are few human studies with adequate statistical power which have examined glucocorticoid – cognitive function relationships. The two largest are considered here. Seeman and co-workers examined the relationship between cortisol levels and cognitive function, both measured at two time points 2.5 years apart, in a sample of 194 healthy men and women aged 70-79 (Seeman et al., 1997). Cortisol levels were measured using 12-hour urinary free cortisols. Cognitive function was assessed with a battery of tests involving immediate and delayed memory tasks, a copying task designed to measure spatial ability, and 'abstraction' (the details of this tasks are not provided). Cross-sectional analyses revealed that in women cortisol secretion was negatively correlated with delayed verbal recall ($r=-0.2$, $p=0.04$); there were no other

significant correlations for the women or the men. Cortisol excretion showed a widening of the variance (that is, a flatter distribution) with a small decrease in the mean excretion for both men and women. In women, there was a significant negative correlation between cortisol increases and delayed paragraph recall ($r=-0.23$, $p=0.01$). There was no such correlation in men. This study did demonstrate the possibility that elevated cortisol might be associated with decrements in cognitive function. However, the population was selected for good health and the age group was comparatively old. This might mean that individuals with the combination of high cortisol and cognitive decrements are selected out before the onset of the study.

Kalmijn and co-workers performed a prospective study of cognitive function and cortisol levels in 189 healthy volunteers aged 55 to 80 who had two sets of measurements two years apart (Kalmijn et al., 1998). Cortisol levels were measured before and after administration of 1mg of dexamethasone. Cognitive function was measured with the MMSE, with subjects being classified as being cognitively impaired if their score was 25 or less, and described as showing cognitive decline if the MMSE score dropped by more than one point. Analyses were conducted after controlling for age, sex, education and depressive symptoms. Using logistic regression an increase of 1 SD in cortisol was associated with only a trend in cognitive impairment but post-dexamethasone cortisol levels were significantly associated with cognitive decline. This study also suggested a possible link between higher cortisol levels and cognitive function despite the use of a relatively insensitive cognitive test.

These studies and a small number of other observational studies lend some support to the hypothesis that there is an association between higher glucocorticoid levels and cognitive function with ageing. However the direction of causation cannot be determined by observational studies. In the next section interventional studies will be reviewed.

Human interventional studies

Most human studies have examined the effects of the administration of cortisol or synthetic glucocorticoids such as dexamethasone or prednisolone over varying periods, from those attempting to mimic the hours-long glucocorticoids seen with stress to several days or weeks of exposure to high doses of glucocorticoids, with varying dosages and different sets of cognitive tests. These studies do not directly address the roles of glucocorticoids in normal human cognitive function and are considered in the next section.

The studies involving synthetic glucocorticoids are difficult to interpret because these drugs probably do not enter the brain because of the *mdr1a* P-glycoprotein (Schinkel et al., 1995). These drugs might actually cause brain levels of glucocorticoid to decrease by inhibiting production of cortisol (which enters the brain freely) via feedback effects on the pituitary. There are also correlational studies which have demonstrated associations among declarative memory and other cognitive functions, stress and cortisol levels (Roy et al., 2001a). However, studies of social stress cannot be used as direct evidence for a causal effect of cortisol on cognitive function because of the complexity of the stress response. There are no published studies examining the effects of blockade of MR or GR on cognitive function in humans.

The first studies reviewed here have examined the effects of short-term increases of cortisol on various cognitive functions. Lupien and co-workers performed a small but interesting study in which four groups of 10 young, healthy men were given placebo, 40, 300, or 600 mg/kg/hr of intravenous hydrocortisone (Lupien et al., 1999). Cognitive function was assessed during the infusions with tests of working memory, declarative memory and a continuous performance task. They found that neither declarative memory nor performance on the continuous performance task was affected in any condition, but that working memory was affected in the highest dose condition. Subjects were only tested during the infusion and unfortunately declarative memory was not assessed after washout, for example, the next day.

In a double-blind, placebo-controlled, crossover trial De Quervain and co-workers examined the effects of acute elevations of cortisol in 36 healthy subjects (18 males and 18 females) aged between 20 and 40 on a declarative memory task (de Quervain et al., 2000). No other cognitive tasks were used. Subjects were shown 60 nouns on a computer screen, one at a time, at intervals of four seconds. 24 hours later they completed free recall and recognition tasks. 25mg cortisone (which is metabolised to cortisol *in vivo*) was given at one of three different times: (A) one hour before the initial presentation, (B) immediately after presentation, or (C) one hour before the recall and recognition tasks. Each subject performed each condition at intervals of two weeks. The report does not state at what time of day the tasks were carried out. Performance was worse on the free recall task in condition C but there were no effects in the other conditions and on the recognition tasks. At the end of condition C, subjects were also administered an immediate free-recall test for an additional immediate free-recall task of 20 words. No effect was observed on performance on this task. The authors interpreted this as showing that stress levels of cortisol impair retrieval but not acquisition or consolidation processes in declarative memory.

Plihal and Born (Plihal and Born, 1999) hypothesised that a drop in cortisol levels overnight is necessary for memory consolidation, based on three pieces of evidence: (1) that slow-wave sleep (as opposed to rapid eye movement sleep or no sleep) facilitates performance on declarative memory tasks, (2) similar patterns of hippocampal neuronal firing during slow wave sleep to those seen during spatial memory learning tasks, and (3) that slow wave sleep tends to occur when glucocorticoid levels are at their lowest. Subjects were 14 healthy men aged between 21 and 31. The study was double-blind placebo-controlled crossover in design. In the first experiment, declarative and non-declarative memory tasks were administered in late evening and after falling asleep subjects were then given either 30mg intravenous cortisol or saline over 3 hours. Then subjects were awoken and performance tested. Cortisol levels increased to a peak of 420 nmol/l in the cortisol infusion condition and dropped to a trough of 51 nmol/l in the placebo condition. Declarative memory but not non-declarative memory was impaired in the cortisol condition. There was no effect on the duration of slow wave sleep. In the second

experiment the authors wished to test the idea that ongoing MR stimulation facilitates memory formation in the hippocampus. 14 similarly aged male subjects were given either 200mg intravenous canrenoate, a specific MR antagonist, over 10 mins at 0900 and 1700, or saline. Interestingly, subjects given canrenoate showed a reduction in slow wave sleep; cortisol levels showed small but significantly different levels (canrenoate: 100 nmol/l; placebo: 33 nmol/l). However, although there were trends in the predicted directions, there were no significant differences in performance on the declarative or non-declarative tasks. The authors interpreted these findings as demonstrating that optimal memory consolidation in slow wave sleep requires GR to be predominantly unoccupied, but that blockade of MR had little effect.

There is another small group of studies which have examined the effects of longer-term elevations of glucocorticoids induced pharmacologically. In a double-blind, placebo-controlled (but not crossover) study Newcomer and co-workers administered placebo, or 40mg or 160mg of cortisol to 51 healthy subjects aged 18 to 30 (Newcomer et al., 1999). Cortisol was given in two divided doses for four days. Cognitive assessments, performed on days one and four of treatment, were paragraph recall (immediate and delayed; oddly the authors only present data on commission errors – ‘intrusions or confabulations’ – and not the standard omission errors), delayed match to sample (spatial memory), the Stroop test, the continuous performance task, verbal fluency and spatial memory. The 160mg cortisol dose produced mean cortisol levels of approximately 750nmol/l, as compared to levels of approximately 200nmol/l for the placebo and 40mg cortisol treatment. The 160mg dose affected performance on both the immediate and delayed components of the paragraph recall task on day four of treatment, but not at day one or performance on any of the other cognitive tasks at day one or four. The 40mg dose had no effect on any cognitive test versus placebo. Performance was similar for all groups after the six day washout period.

Young and co-workers administered placebo or cortisol 20mg twice a day for 10 days to 20 males aged between 21 and 44 (Young et al., 1999). Subjects were given

both treatments with a three-week washout period. Significant differences were found in tasks of spatial working memory and delayed spatial memory. No deficit was observed in the Tower of London task (a test of 'executive' function).

Few studies have studied the effects of long-term administration of glucocorticoids on cognitive function in humans. Keenan and co-workers (Keenan et al., 1996a) examined cognitive function in a group of 25 patients who had been taking between 5 and 40mg of prednisolone for at least 1 year compared with 25 controls (age-matched patients with similar diagnoses). They found that the patients performed worse than the controls on a test of delayed verbal memory but showed the same performance on tests of procedural memory, fluid intelligence and other cognitive tests.

The above studies, though interesting, are difficult to interpret because of several reasons, including the generally low statistical power and the different paradigms used. In the light of the animal studies demonstrating that situational and temporal context is crucial in determining the effects of short-term fluctuations in glucocorticoids perhaps the only clear conclusion from the human studies is that giving high doses of glucocorticoids for sustained periods (that is, for longer than the duration of a typical stress response), or in such a way as to override circadian rhythms, probably reduces performance on some cognitive tasks. In animals, performance on tasks known to be dependent on intact hippocampi is often affected. In humans the number of studies is too small to determine if any there are specific effects on tasks involving immediate and delayed memory.

1.4.4 Conclusions

Currently there is enough evidence to suggest that some ageing animals and humans develop dysregulation of the HPA axis. In most studies this is manifested by a slower return to normal of glucocorticoids following an episode of stress but some studies have shown a flattening of the distribution curve of overall output of glucocorticoids, with the result that some ageing animals and humans do show relative hypersecretion of glucocorticoids. However, there are surprisingly few informative studies in humans; the two published studies of sufficient size have only had follow-up periods of two to three years.

The pathophysiology of ageing-related changes in the HPA axis is unclear. Although the main sites of HPA axis feedback are the pituitary gland and the hypothalamus, the hippocampus and other sites in the brain also exert influence on the HPA axis. The hippocampus exerts an inhibitory effect on the HPA axis: lesions of the hippocampus cause rises in glucocorticoid levels. Both MR and GR are thought to play a role in this. The significance of ageing-related hippocampal atrophy and changes in receptor densities and other determinants of glucocorticoid sensitivity such as 11β -HSDs in this context are as yet unclear.

That prolonged elevations of glucocorticoids result in changes in the brain is in little doubt. Animal studies have demonstrated that interventions which result in an increase in exposure of the brain to glucocorticoids cause atrophy of the hippocampus, particularly in the CA3 field. Landfield's important interventional study (Landfield, 1981) in which ageing-associated elevations of corticosterone in rats were prevented by adrenalectomy and low-dose replacement provided strong initial evidence and multiple studies have confirmed varying degrees of atrophy following prolonged exposure to high levels of glucocorticoids. In humans, the strongest evidence undoubtedly comes from the brain atrophy observed in Cushing's syndrome.

The evidence directly linking elevated glucocorticoids with cognitive impairments in animals is perhaps less clear-cut. Some observational studies, in particular that of

Issa and co-workers (Issa et al., 1990), have provided evidence suggesting that there is an association between rising glucocorticoid levels and cognitive impairment. However, not all interventional studies have shown such an association. Some indirect evidence has emerged from the antidepressant studies of Yau and co-workers (for example, (Yau et al., 2002), which have shown that antidepressant treatment attenuates ageing-related cognitive impairments. The effects on cognitive function are paralleled by lower overall output of corticosterone in the treated animals, and an explanation for this effect is provided by the relatively higher levels of MR and GR in the hippocampus of the treated animals, which might enhance feedback regulation of the HPA axis. Further and more direct evidence is provided by the recent development of mice with the gene for 11 β -HSD1 (which regenerates active corticosterone from deoxycorticosterone) knocked out; these mice show striking attenuation of ageing-related cognitive deficits in comparison to controls.

In humans very strong evidence of the adverse effects of prolonged elevated glucocorticoids on cognitive function is provided by several studies demonstrating widespread cognitive deficits in Cushing's syndrome. Observational studies with sufficient power are lacking but those that do exist have shown small associations between glucocorticoid levels and ageing-related cognitive decrements.

1.5 Hyperglycaemia and cognitive ageing

A key feature of ageing is the increasing degree of carbohydrate intolerance observed (Arking, 1998). There are several methods of measuring this, including fasting glucose levels, glucose levels after a carbohydrate (usually glucose) load, and also levels of glycosylated haemoglobin (HbA_{1c}). HbA_{1c} levels reflect the average blood glucose concentration over a 6-8 week period and are used in diabetic patients to monitor blood glucose control.

Type II diabetes mellitus (DM) is defined by the American Diabetic Association as fasting glucose greater than or equal to 7.0 mmol/l, and the intermediate category of 'impaired fasting glucose' as a fasting glucose of 6.1 to 6.9 mmol/l (Harris et al., 1998). Associated with hyperglycaemia is the 'metabolic syndrome'. An individual is said to have the metabolic syndrome if they have three or more of the following abnormalities: waist circumference greater than 102 cm in men and 88 cm in women; serum triglycerides level of at least 1.69 mmol/l; high-density lipoprotein cholesterol level of less than 1.04 mmol/l in men and 1.29 mmol/l in women; blood pressure of at least 130/85 mm Hg; or serum glucose level of at least 6.1 mmol/l. Although not a part of the formal definition of the metabolic syndrome, insulin resistance is also common, with higher fasting insulin levels clustering with these abnormalities.

The incidence of all of these abnormalities increases with age, and therefore the number of people with the metabolic syndrome is rising. In the United States the prevalence of the metabolic syndrome is now 40% in people over the age of 60. The metabolic syndrome is known to be an important cardiovascular risk factor (Ford et al., 2002). Recently there is growing interest in the role that type II DM, hyperinsulinaemia and components of the metabolic syndrome might have in determining age-related cognitive decrements.

Type II DM is an established risk factor for Alzheimer's disease (Ott et al., 1999) and also for vascular dementia (Hebert et al., 2000). However, it is also associated with milder cognitive impairments. Strachan and co-workers reviewed the literature

in 1997 and found that of 17 published studies, 13 showed an association of diabetes mellitus with milder cognitive impairment, most often affecting tests of verbal memory (Strachan et al., 1997). Since then other studies have largely confirmed this finding (MacKnight et al., 2002).

In keeping with the associations with ageing-related cognitive impairment type II DM is probably associated with changes in the brain, though the number of studies is small. Animals with streptozocin-induced diabetes (in which rats are injected with streptozocin, causing damage to insulin-producing pancreatic beta-cells) show deficits in processes underlying memory formation in the hippocampus (Biessels et al., 2002). In humans, two small studies have linked type II DM with cerebral atrophy (Soininen et al., 1992; Araki et al., 1994).

Type II DM is well known to be associated with increased risk of vascular disease, including stroke and cardiovascular disease. Recently there have been attempts to link evidence of relative carbohydrate intolerance in subjects not meeting the criteria for diabetes mellitus, for example subjects with impaired fasting glucose or elevated HbA1c. In a prospective study of 4662 men Khaw and co-workers found that in HbA1c levels were continuously related to mortality from all-cause and cardiovascular causes in both diabetics and non-diabetics (Khaw et al., 2001). This effect was independent of age, blood pressure, serum cholesterol, body mass index, and smoking. There are no current studies linking HbA1c with cognitive function in non-diabetics, but other related variables have been studied, including hyperinsulinaemia (Kuusisto et al., 1993), hyperlipidaemia (Kivipelto et al., 2001) and hypertension (Meyer et al., 1999; Meyer et al., 2000; Starr, 1999; Swan et al., 1998), with most studies finding small but significant associations.

1.6 Aims and Hypotheses

This thesis has several aims. Firstly, the relationships among performance on different cognitive tests are analysed, with the hypothesis that the tests will be significantly and positively intercorrelated. Principal components analyses will be used to derive latent traits. Secondly, detailed volumetric and spectroscopic neuroimaging is used to characterise volumes of certain brain regions (hippocampi, temporal lobes and frontal lobes) and metabolite levels. Thirdly, relationships among cognitive and neuroimaging variables are examined. Specifically the hypotheses that larger brain volumes and that higher levels of N-acetylaspartate are associated with better performance on cognitive tests are tested. Fourthly, the hypotheses that increased glucocorticoid levels are associated with (a) relative atrophy of the hippocampi, (b) lower N-acetylaspartate levels and (c) relative cognitive impairment are tested. Finally, the hypothesis that hyperglycaemia is associated with relative cognitive impairment is tested.

Chapter 2: Methods

2.1 Recruitment

Subjects were 111 healthy, unmedicated male volunteers aged 65-70. They were recruited with the help of local general practitioners in several practices in Edinburgh. After the initial approach by means of an explanatory letter, general practitioners were asked to provide a list of men aged 65 to 70 in their practice who were not known to have significant illness and who were not taking regular medication. Once this list was supplied, I visited each practice and screened the casenotes of the men on the list, looking for evidence of ill health or regular medication. Once these notes had been screened, letters with stamped, addressed envelopes were sent to subjects asking if they would agree to being telephoned to hear more about a research study.

Of those written to, about half in each practice replied positively. These volunteers were then telephoned and the basics of the study were explained. If they continued to show interest then I conducted a brief telephone screening questionnaire asking the volunteer about serious illness, attendance at their general practice or at hospital outpatient clinics, alcohol intake (those with an intake of greater than 28 units per week were excluded), and use of prescribed, regular medication. If the screening tests were negative then a visit to the volunteer's home was arranged. In the home visit a more detailed screening of symptoms was taken and any subjects with evidence of illness were excluded and referred to their general practitioner. This included, for example the subject describing angina or symptoms of depression or anxiety. If the medical screening was negative I then went on to describe the study in greater detail and obtain consent.

Age, body mass index and waist-hip data are shown in Table 2.1. As might be expected in a group of healthy elderly volunteers, social classes I and II were

overrepresented in comparison to the population: 33.9% (N=39) were in social class I, 33% (N=38) were in social class II, 18.3% (N=21) were in social class III, 10.4% (N=12) were in social class IV and 0.9% (N=1) in social class V.

Table 2.1: Age, Body Mass Index and Waist-Hip Ratio data

	Mean	Range	Standard Deviation
Age (years)	67.8	65.4 – 70.4	70.4
Body Mass Index	26.9	20.4 – 34.6	2.94
Waist-Hip Ratio	0.94	0.80 – 1.11	0.057

2.2 Cognitive tests

These tests were selected on the basis that they are well-established, reliable and valid. Also, none has a strong ceiling effect, that is, few or none of the subjects will score maximum points. The tests are thought to test different cognitive domains, and, other than the National Adult Reading Test, show substantial decrements with ageing (Lezak, 1995). Because of hypotheses concerning the effects of glucocorticoids on medial temporal lobe structures, and because damage to medial temporal lobe structures is known to be associated with decrements in tests involving recall of previously-presented information, there is an emphasis on assessments of memory in the selection of the tests.

'Premorbid' or 'prior' IQ

National adult reading test

The National Adult Reading Test (NART) (Nelson and Willison, 1991) is a test in which the subject is asked to pronounce irregularly spelt words such as "chord", "ache" and "leviathan". This is thought to indicate familiarity with the word and thus is related to a subject's vocabulary. Performance on this test correlates highly with full scale IQ as measured by the Wechsler Adult Intelligence Scale (Lezak, 1995). In healthy populations scores on the NART correlate more highly with verbal IQ than with performance IQ (Crawford et al., 1989). The main use of the NART is as a means of estimating 'premorbid' IQ, as scores correlate highly with IQ scores in childhood and adulthood (Crawford et al., 2001), though the term 'prior' IQ is perhaps preferable in ageing populations without specific disease.

Verbal memory

Auditory-verbal learning test (AVLT)

The auditory-verbal learning test (AVLT) (Lezak, 1995) involves reading out a list of 15 words at a rate of one per second, then asking the subject to repeat as many words back as possible (Appendix 2). The examiner then reads out the same list at the same rate and the subject then attempts to repeat back the same list, including words previously recalled. This procedure is repeated until the list is read a total of five times. Then a different list of words is read once and the subject is asked to repeat as many as he or she can from the new list. After this, the first list is read out again and the subject is asked to again recall as many words as possible from the first list. There then follows a delay of 30 minutes, after which the subject, without having the original list read out again, is asked to recall as many words from the first list as possible.

Logical Memory

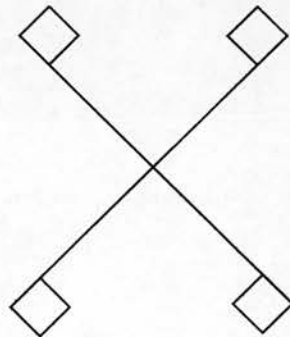
Logical Memory is one of the subtests from the Wechsler Memory Scale – Revised (Wechsler, 1981). This is a test involving the presentation of two short stories with 25 scoring units or ‘memorable ideas’. The examiner stops after each story and asks the subject for immediate free recall. After a delay of 30 minutes the subject is asked to recall as much of each story as possible. In the current studies, some of the subjects were also asked to return the following day for testing of free recall of Logical Memory; this is termed ‘Logical Memory – 24hr delayed’. Ageing and dementia are both associated with reduced scores in both the AVLT and Logical Memory (Lezak, 1995; Mitrushina et al., 1991; Abikoff et al., 1987; Storandt et al., 1984).

Visuospatial memory

Visual Reproduction

This test is also part of the Wechsler Memory Scale – Revised (Wechsler, 1987). In this test the subject is shown a series of four simple geometric designs. An example is shown in Figure 2.1. A subject is given five seconds to look at one design, then this is hidden and the subject is asked to draw the design. The test is repeated, without further prompting, 30 minutes after the initial presentation. Performance on Visual Reproduction declines with age and with dementia (Wilson et al., 1983; Bak and Greene, 1981; Lezak, 1995).

Figure 2.1: Sample item from Visual Reproduction

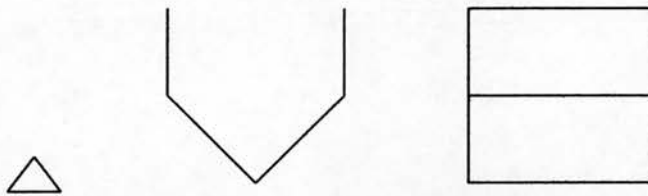


Benton Visual Retention Test

The Benton Visual Retention Test (BVRT) is a test of visuospatial recall (Sivan, 1992). The subject is shown a series of 10 cards with between one and three simple geometric designs on each. An example is shown in Figure 2.2. The subject is asked to look at the card for 10 seconds, then the card is hidden and the subject has to wait five seconds before reproducing the shapes on a blank sheet of paper. This method is

known as Administration B. Each item was scored either 0 or 1. As with Visual Reproduction, performance on the BVRT declines with age and dementia (Giambra et al., 1995; Lezak, 1995).

Figure 2.2: Sample item from the Benton Visual Retention Test



Verbal Fluency

The most commonly-used test of verbal fluency is the Controlled Oral Word Association Test (Lezak, 1995). The examiner speaks out loud a letter from the alphabet and the subject is instructed to speak out loud as many words beginning with that letter as possible in one minute, excluding proper nouns and the same word with different suffixes. In this thesis, the letters C, F and S were used. This test has been shown to be sensitive to damage to the frontal lobe (Lezak, 1995) and is often considered a test of 'executive' or 'frontal lobe' function. As with the above tests, performance on the Controlled Word Association Test shows decline with ageing and also in dementia (Lezak, 1995).

Non-verbal reasoning

Raven's Standard Progressive Matrices

Raven's Standard Progressive Matrices (RSPM) consists of a series of visual pattern matching and analogy problems shown in abstract designs (Raven et al., 1977).

RSPM is thought to be one of the best tests of fluid intelligence and loads highly on to the general cognitive factor (Carroll, 1993). There are 60 items organised in five groups of 12. Subjects were given 20 minutes to complete the test and the score was the number correct. Performance on RSPM shows decline with ageing (Carlson and Jensen, 1981) and with dementia (Gainotti et al., 1992).

Attention and Processing Speed

Digit Symbol

The Digit Symbol Substitution Test (DSST) of the Wechsler Adult Intelligence Scale-Revised (Wechsler, 1981) was used as an indicator of attention and processing speed. Subjects are instructed to draw in symbols in blank boxes under numbers according to a code given at the top of the answer sheet. The score was the number of correct substitutions in 90 seconds. The DSST is highly sensitive to age (Wechsler, 1981), showing declines from the age of 20, and also shows decline in dementia (Lezak, 1995).

2.3 Testing protocol

Subjects were asked to fast overnight before attending, and then either arranged their own transport or were brought in a taxi to the Western General Hospital. Blood was taken for cortisol, glucose, full blood count, B12 and folate, urea and electrolytes, total cholesterol and thyroid function tests. Once the blood was taken, subjects were given breakfast in the hospital canteen. Subjects were given decaffeinated tea and coffee.

After 30 minutes, subjects were brought to the cognitive testing room and the protocol started. The following tests were administered:

National Adult Reading Test, Digit-Symbol Substitution Test, Auditory-Verbal Learning Test, Raven's Matrices, Logical Memory, Visual Reproduction, and the Benton Visual Retention Test. The delayed components of the Auditory-Verbal Learning Test, Logical Memory and Visual Reproduction were administered 30 minutes after the immediate components. The tests were administered with a standardised sequence of breaks which allowed the tests of the delayed components to be administered at 30 minutes. The length of the testing session was two-and-half hours and there were three breaks.

Subjects were then given lunch and returned at 2.30pm to have blood taken for cortisol. The following day a proportion of subjects returned to have recall on Logical Memory re-tested.

At least one week later subjects were asked to take dexamethasone 0.25mg at 11pm and come to the Western General Hospital the following morning (0900) to have blood taken for cortisol measurement.

2.4 Blood sampling

Levels of plasma glucose, HbA1c and other standard clinical tests were carried out by the Clinical Biochemistry department in the Western General Hospital. Glucose levels were measured with a Roche Hitachi 912 using the enzymatic method; the between-batch precision for this assay is 1.4%. HbA1c levels were measured with a BioRad Variant 2 using ion-exchange high performance liquid chromatography; the between-batch precision for this assay is 1.2% (details of the glucose and HbA1c assays were provided by Dr. Susan Walker of the Clinical Biochemistry department). Blood samples for cortisol were immediately spun and frozen at -20°C . The radioimmunoassay for cortisol levels is described in Chapter 6.

2.5 Magnetic resonance imaging and spectroscopy

Subjects were scanned in a 1.9T Elscint scanner in the Brain Imaging Research Centre for Scotland in the Department of Clinical Neurosciences in the Western General Hospital. Scanning protocols allowed for detailed volumetric imaging, imaging of white matter lesions and magnetic resonance spectroscopy.

Neuroimaging data reported here are in two categories: brain and intracranial volumes and spectroscopy. As the scanning protocols and methods of analysis were designed and carried out by staff in the Brain Imaging Research Centre for Scotland, these methods are in Appendix 1.

2.6 Statistical analyses

All statistical analysis was carried out using SPSS versions 10 and 11 apart from the structural equation modelling, which was carried out by Professor Ian Deary using the EQS program. Specific analyses are described in each chapter.

Chapter 3: Cognitive function data

3.1 Introduction

Because the cognitive data apply to all of the remaining data chapters, an overview of the data is provided here. First, basic descriptive data are provided, then the data are reduced by means of correlational and principal components analyses. These latter two procedures were carried out because in much of the literature relationships among scores on cognitive tests and other types of variables, such as brain volumes, are analysed without accounting for shared variance among cognitive test scores. Overall, 111 men were tested but because 97 had usable scans, the data from these 97 are described here.

3.2 Descriptive Statistics

Table 3.1 shows the minimums, maximums, means and standard deviations for the data. N= 97 for all tests except N=96 for Logical Memory and Visual Reproduction, in which two different subjects had their testing session interrupted, Raven's Standard Progressive Matrices (RSPM) (N=95), in which two subjects did not complete their answer sheets correctly, and Logical Memory 24hr, in which 59 subjects returned the following day for testing of recall.

Table 3.1: Descriptive data for cognitive tests

Cognitive Test	N	Minimum	Maximum	Mean	SD
NART	97	13	50	36.2	9.3
Verbal Fluency	97	17	85	43.4	13.1
DSST	97	23	73	48.4	10.5
AVLT trials 1 to 5	97	30	66	49.1	7.8
AVLT trial 7	97	7	15	11.7	2.0
BVRT	97	0	10	6.7	2.0
Logical Memory 1	96	10	42	25.3	6.1
Logical Memory 2	96	4	40	20.9	6.9
Logical Memory 24hr	59	5	33	18.7	6.7
Visual Reproduction 1	96	19	41	33.5	5.2
Visual Reproduction 2	96	8	41	27.5	7.8
RSPM	95	11	59	41.5	8.6

Abbreviations: **NART:** National Adult Reading Test; **DSST:** Digit-Symbol Substitution Test; **AVLT1_5:** summed scores from the Auditory Verbal Learning Test trials 1 to 5; **AV7:** Auditory-Verbal Learning Test trial 7; **AVLT:** Auditory-Verbal Learning Test; **BVRT:** Benton Visual Retention Test; **RSPM:** Raven's Standard Progressive Matrices.

Data from some of the tests (National Adult Reading Test, the Benton Visual Retention Test and Visual Reproduction) showed some skew to the right (histograms

for all the tests are shown in Appendix 2). However, this was not so severe as to preclude the use of parametric statistical tests.

3.3 Correlations

Studies have shown scores from the immediate and delayed components of memory tests are often very highly correlated. Therefore these components of Logical Memory, Visual Reproduction and the AVLT were correlated. These correlations are shown in Table 3.2.

Table 3.2. Correlations between immediate and delayed components of memory tests

Tests	Correlation (Pearson)
Logical Memory 1 <i>versus</i> Logical Memory 2	$r=0.86$, ($p<.0001$, $N=96$)
Visual Reproduction 1 <i>versus</i> Visual Reproduction 2	$r=0.78$ ($p<.0001$, $N=96$)
AV1_5 <i>versus</i> AV7	$r=0.64$ ($p<.0001$, $N=97$)

Abbreviations: **AV1_5:** summed scores from the Auditory Verbal Learning Test trials 1 to 5; **AV7:** Auditory-Verbal Learning Test trial 7.

Because these correlations are high and because of the high degree of overlap in terms of constructs, and also because of concerns over multicollinearity when multiple regression and other multivariate techniques are used, the scores from each of these pairs of immediate and delayed memory tests were standardised and summed. In the rest of this thesis, the variables Logical Memory, Visual Reproduction and AVLT refer to these summed scores.

The data were then explored in terms of patterns of correlations, using initially Pearson's correlations and then principal components analysis. The correlation matrix for the cognitive tests is shown in Table 3.3. This shows that all of the tests are positively and significantly intercorrelated. However, it is clear that some tests are more related than others. For example, AVLT correlates at $r=0.56$ with Logical Memory, another test of verbal memory, whereas it correlates at $r=0.30$ with the Benton Visual Retention Test, a test of visuospatial memory. These findings prompt the use of principal components analysis to uncover the underlying structure of the relationships among these tests.

Table 3.3. Pearson correlations among cognitive tests

	VF	DSST	AVLT	LM	LM 24	VR	BVRT	RSPM
NART	.52**	.52**	.32**	.44**	.36**	.42**	.42**	.44**
VF		.42**	.27**	.27**	.29*	.35**	.38**	.35**
DSST			.37**	.42**	.38**	.54**	.48**	.57**
AVLT				.56**	.64**	.34**	.30**	.35**
LM					.94**	.46**	.31**	.32**
LM 24						.48**	.47**	.37**
VR							.61**	.63**
BVRT								.65**

** $p < .01$; * $p < .05$ (2-tailed). Listwise deletion.

Abbreviations: **NART:** National Adult Reading Test; **VF:** verbal fluency; **DSST:** Digit-Symbol Substitution Test; **AVLT:** Rey Auditory-Verbal Learning Test; **BVRT:** Benton Visual Retention Test; **LM:** Logical Memory; **LM 24:** Logical Memory – 24hr delayed; **VR1:** Visual Reproduction; **RSPM:** Raven's Standard Progressive Matrices.

3.4 Principal Components Analyses

Principal components analysis is a technique used, amongst other things, to explore the relationships among variables and to determine if relationships among variables can be summarised by a smaller number of variables, termed ‘components’ (in the case of principle component analysis, ‘latent variables’ (a general term) or ‘factors’ (a term used in the related technique of factor analysis) (Norman and Streiner, 2000). In this thesis the term ‘factor’ is used, because it has now entered into common usage for both principal components analysis and factor analysis. Principal components analysis is useful when the data represent multiple measurements of features of a system which might be related. For example, a group of individuals have the lengths of the bones in their limbs measured. These measurements would probably show multiple positive intercorrelations; principal components analysis could be used to determine whether these intercorrelations are best represented by one large factor. This technique could also be used to determine if certain variables cluster together more than others, for example, whether lengths of bones in the fingers showed intercorrelations higher than the general positive correlation among all the bones.

Principal components analysis was performed on the following cognitive tests: Verbal Fluency, Digit-Symbol Substitution Test, AVLT, BVRT, Logical Memory, Visual Reproduction and Raven’s Standard Progressive Matrices (n=95). The National Adult Reading Test was excluded from this analysis because it is a measure of premorbid or prior IQ. Logical Memory 24hr - delayed was also excluded because of clear overlap between it with Logical Memory scores and also because the number of subjects (N=59) was substantially lower.

There were two components with eigenvalues of greater than one. The first unrotated component accounted for 51.3% of the total variance, which represents the typical amount of test covariance accounted for by the general cognitive factor or ‘g’ (referred to in Chapter 1: Introduction). The second component accounted for 14.5% of the total variance. A scree plot of this analysis is shown in Figure 3.2. Although

there were two components with eigenvalues greater than one, the scree plot suggests one significant component. Loadings (analogous to beta-weights in a multiple regression analysis) for each test score on the two components are shown in Table 3.4.

Figure 3.2. Scree plot for the unrotated principal components analysis of cognitive tests

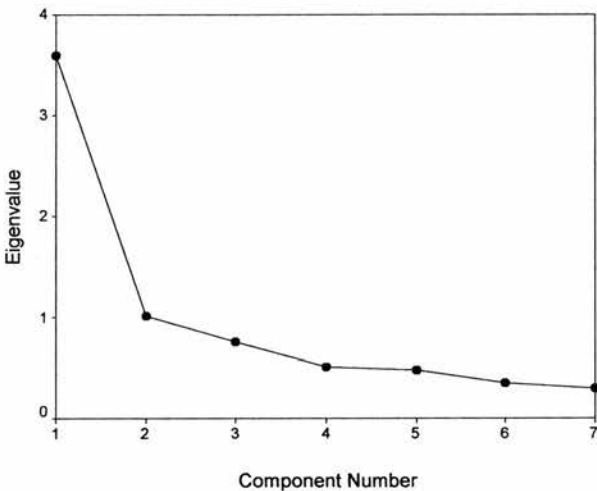


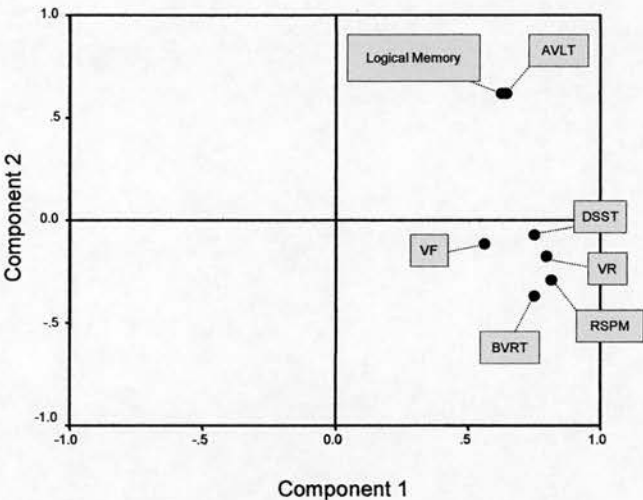
Table 3.4. Loadings on the first two unrotated components from the unrotated principal components analysis

	Component 1	Component 2
Verbal Fluency	.56	-.11
Digit-Symbol Substitution Test	.76	-.01
Auditory-Verbal Learning Test	.63	.61
Logical Memory	.65	.61
Visual Reproduction	.81	-.18
Benton Visual Retention Test	.75	-.38
Raven's Matrices	.81	-.30

Rotation

Table 3.4 shows that Verbal Fluency, the Digit-Symbol Substitution Test, Visual Reproduction, the Benton Visual Retention Test and Raven's Standard Progressive Matrices all show positive loadings on to the first component, and either zero or negative loadings on the second component. Logical Memory and the Auditory-Verbal Learning Test show approximately equal loadings on both components. These loadings are plotted in Figure 3.3.

Figure 3.3. Plot of loadings of individual tests on the first and second unrotated components from the principal components analysis



Abbreviations: **AVLT:** Auditory-Verbal Learning Test; **VF:** verbal fluency; **DSST:** Digit-Symbol Substitution Test; **VR:** Visual Reproduction; **BVRT:** Benton Visual Retention Test; **RSPM:** Raven's Standard Progressive Matrices.

These results suggest that it might be appropriate to rotate the axes. One aim of rotation is to achieve ‘structural simplicity’ (Norman and Streiner, 2000) (p. 171). Rotation is carried out when, for example, the variance is unequally distributed, and/or when some variables load strongly on more than one factor. In the present case, Logical Memory and the Auditory-Verbal Learning Test show strong loadings on two different components. Therefore rotation was carried out, using the Direct Oblimin method, which allows for correlated (oblique) factors.

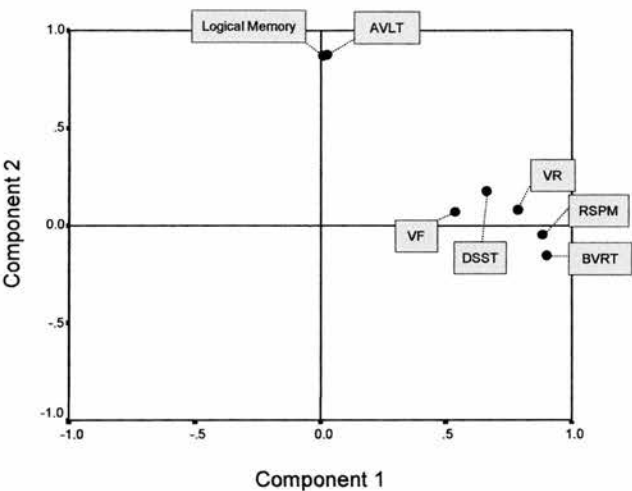
This yielded two correlated components ($r=0.47$). The loadings for these components are shown in Table 3.5, and the plot of these loadings in Figure 3.4. Component 1 has higher loadings from Verbal Fluency, the Digit-Symbol Substitution Test, the

Benton Visual Retention Test, Visual Reproduction and Raven’s Standard Progressive Matrices. Component 2 has larger loadings from the Auditory-Verbal Learning Test and Logical Memory. Component 1 seems to reflect tests involving visuospatial memory and processing, and also tests involving an element of the poorly-understood but empirically prominent construct of processing speed. Component 2 reflects verbal memory.

Table 3.5. Pattern matrix for rotated principal components analysis

	Component 1	Component 2
Verbal Fluency	.54	.07
Digit-Symbol Substitution Test	.66	.18
Auditory-Verbal Learning Test	.01	.87
Logical Memory	.03	.88
Visual Reproduction	.79	.08
Benton Visual Retention Test	.90	-.16
Raven’s Matrices	.88	-.05

Figure 3.4. Plot of loadings of individual tests on the first and second rotated components from the principal components analysis



Abbreviations: *AVLT*: Auditory-Verbal Learning Test; *VF*: verbal fluency; *DSST*: Digit-Symbol Substitution Test; *VR*: Visual Reproduction; *BVRT*: Benton Visual Retention Test; *RSPM*: Raven's Standard Progressive Matrices.

3.6 Conclusions

In summary, tests showed a wide spread of variance. Scores were normally distributed apart from the National Adult Reading Test and the Benton Visual Retention Test, which were skewed towards the right (that is, higher scores). This might indicate that the sample has a higher average overall cognitive ability than the general population. Because these tests tend to show normal distributions in larger samples, they were treated as if they had normal distributions.

Correlational analyses first showed that the immediate and delayed components of the memory tests were highly correlated and for the reasons given above these components were combined to give a single variable representing performance on each test. Correlations between all tests were universally significant and positive, indicating a substantial amount of shared variance. Because of this, cognitive test scores were subjected to data reduction with principal components analysis. This showed initially that 51.5% of the total variance was common, in keeping with findings summarised by Carroll (Carroll, 1993) and other investigators (Deary, 2001a). Rotation revealed that to some extent a visuospatial processing / fluid factor could be distinguished from a verbal memory factor.

Chapter 4: Cognitive function and brain volumes

4.1 Introduction

In investigating possible neuroanatomical substrates of ageing-related cognitive decline, magnetic resonance imaging (MRI) and cognitive function studies in elderly people predominantly focus on relationships between ageing-related changes in specific brain regions and specific cognitive functions, such as hippocampal volume and declarative memory (Lupien et al., 1998). However, cognitive ability differences are stable over several decades (Deary et al., 2000b) and, in young, healthy adults, brain size is associated with individual differences in cognitive function (Vernon et al., 2000). Therefore, brain structure-cognitive function relationships observed in elderly people may be due not only to the effects of ageing-associated atrophy, but also to baseline differences in cognitive functioning.

Estimates of young-adult brain size in elderly people can be derived from intracranial volume. The cranial vault is virtually fixed by the age of seven, and brain size closely approximates this until the sixth or seventh decades (Sgouros et al., 1999; Mori et al., 1997). Intracranial volume is important in helping to estimate the degree of brain atrophy with ageing. It is also of interest as a measure in itself because, if intracranial volume is associated with cognitive function in older people, this suggests that genetic and early life factors may be important in determining variations in cognitive function in late life (Brandt et al., 1993; Vallee et al., 1999).

Another important issue is that, in health, performances on diverse cognitive tests show virtually universal positive correlations (Carroll, 1993; Carroll, 1993; Deary, 2001a). Factor analysis can be used to derive a measure of the common variance in a set of cognitive tests; this general factor is often termed *g* (Neisser et al., 1996). Performance on a test of memory is thus influenced by *g* and test-specific variance (Tremont et al., 1998a). Because of the association between brain size and IQ in

young adults, it may be that, in elderly people, relationships between regional brain volumes and certain tests are mediated partially by associations between overall brain volume and g .

In this study the hypothesis that intracranial volume and regional brain volumes are positively associated with cognitive function is tested. To determine whether there were specific relationships between specific brain regions and particular cognitive tests, or whether any relationship was due to overall relationships between overall brain size and overall cognitive ability, structural equation modelling was used.

4.2 Methods

Subjects and cognitive testing were as described in Chapter 2: methods.

4.2.1 Neuroimaging

Neuroimaging analyses were conducted by Dr. Karen J. Ferguson (appendix 1). These analyses yielded data for intracranial area (ICA), hippocampal volumes, temporal lobe volumes and frontal lobe volumes. Intracranial area was used as an estimate for intracranial volume. Dr. Ferguson measured intracranial volume in 40 subjects, blind to the values for ICA, and this correlated at $r=0.88$ ($p<0.001$) with ICA. ICA is therefore a good estimate of intracranial volume.

4.2.2 Statistical Analysis

Descriptive statistics for the neuroimaging data were generated, then cognitive test scores were correlated with each other and with MRI data (including brain volumes, unadjusted and adjusted for ICA (created by saving residuals from a linear regression)) using Pearson's correlation coefficient. Data reduction was performed using principal components analysis as described in Chapter 3. Structural equation modelling was used to test competing models of the association between brain volumes and cognitive functions³.

³ Structural equation modelling was performed by Professor Ian Deary

Figure 4.1: Intracranial area



4.3 Results

Of the 100 participants scanned, three were excluded when unexpected pathology on structural MRI was discovered: two because of congenital arachnoid cysts, and one because of a pituitary adenoma.

The descriptive statistics for the regional brain volumes and ICA are presented in Table 4.3. All brain volumes and ICA were normally distributed. Regional brain volumes were correlated significantly and positively, with right and left sides of the same region more strongly correlated than different regions. ICA was significantly associated with all the brain regions measured (Table 4.4).

Table 4.5 shows the correlation matrix between the cognitive tests, and regional brain volumes and ICA. This shows that standard intelligence tests, including the National Adult Reading Test, the Digit-Symbol Substitution Test and Raven's Standard Progressive Matrices, and tests of visuospatial memory were significantly and positively correlated with several brain region volumes. By contrast, tests of verbal memory and verbal fluency did not correlate significantly with any brain volumes. When brain volumes were adjusted for ICA, only the correlation between right temporal lobe volume and Visual Reproduction remained significant.

Table 4.3: Descriptive statistics for regional brain volumes and intracranial area (ICA)

	Mean	Median	Minimum	Maximum	SD
Left hippocampus (mm ³)	3500	3491	2111	4580	439.5
Right hippocampus (mm ³)	3626	3637	2582	5196	465.5
Left frontal lobe (mm ³)	61220	60174	44313	88566	8911.6
Right frontal lobe (mm ³)	65041	65000	38067	108350	11497.6
Left temporal lobe (mm ³)	68408	68591	46979	85115	7878.7
Right temporal lobe (mm ³)	72928	73545	48599	99254	8649.3
ICA (mm ²)	15923	16011	14005	19095	986.8

Table 4.4: Pearson correlations among brain volumes and intracranial area (ICA)

	Right hippo- campus	Left frontal lobe	Right frontal lobe	Left temporal lobe	Right temporal lobe	ICA
Left hippocampus	.83**	.40**	.33*	.59**	.52**	.39**
Right hippocampus		.41**	.39**	.46**	.54**	.44**
Left frontal lobe			.75**	.46**	.39**	.45**
Right frontal lobe				.29*	.49**	.50**
Left temporal lobe					.75**	.43**
Right temporal lobe						.51**

* $p < .005$; ** $p < .000$, 2-tailed. N=97.

Table 4.5: Pearson correlations among regional brain volumes, intracranial area (ICA), and cognitive tests

	Left hippo- campus	Right hippo- campus	Left frontal lobe	Right frontal lobe	Left temporal lobe	Right temporal lobe	ICA
NART	.07	.11	.23 [*]	.16	.06	.17	.30 [†]
VF	-.02	.06	.15	.11	-.07	.16	.16
DSST	.23 [*]	.21 [*]	.26 [*]	.16	.17	.21 [*]	.32 [†]
AVLT	.01	.00	.02	.00	-.10	-.03	-.01
LM	.04	.10	.06	.02	-.04	.06	.12
VR	.23 [*]	.21 [*]	.19	.18	.19	.33 [†]	.27 ^{**}
BVRT	.10	.09	.23 [*]	.22 [*]	.11	.20 [*]	.26 ^{**}
RSPM	.23 [*]	.20 [*]	.22 [*]	.25 [*]	.22 [*]	.32 [†]	.39 ^{††}

*p<.05; **p<.01; †p<.005; ††p<.000, 2-tailed. N=97 except for RSPM (N=95), LM (N=96), and VR (N=96).

Abbreviations: **NART:** National Adult Reading Test; **VF:** verbal fluency; **DSST:** Digit-Symbol Substitution Test; **AVLT:** Auditory-Verbal Learning Test; **LM:** Logical Memory; **VR:** Visual Reproduction; **BVRT:** Benton Visual Retention Test; **RSPM:** Raven's Standard Progressive Matrices

Data reduction

Principal components analysis

Because of the large amount of shared variance throughout the different brain regions and across different cognitive domains these variables were subjected to data reduction. Principal components analysis of the right and left hippocampi, temporal lobes and frontal lobes revealed that the first unrotated component accounted for 59% of the total variance ($N=97$). Thus, individual differences in these specific brain areas reflect, to a large degree, differences in overall brain size. Principal components analysis was performed on the cognitive tests as described in Chapter 3.

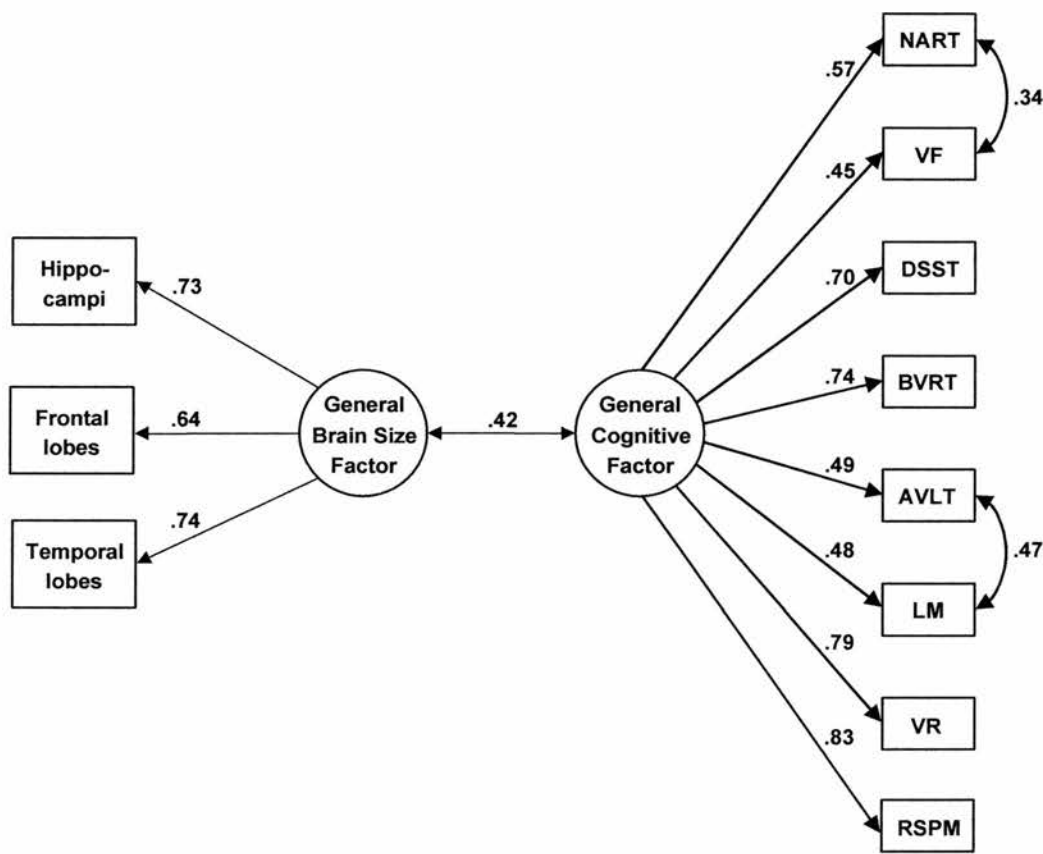
Structural equation modelling

The model applied to the present data is illustrated in Figure 4.2. The arrows represent the relationships between the measured variables and the derived latent traits, and also between the two latent traits. Numbers beside arrows that connect variables are parameter weights estimated by the EQS program: squaring them indicates the variance shared by adjacent variables. Because of the high correlations between volume measurements from the right and left sides, these were reduced (via summed standardised scores) to a single value for each brain region. The three brain region volumes were hypothesised to load on a general brain volume latent trait. The eight cognitive test variables were hypothesised to load on a single general cognitive latent trait. The correlation between these two latent traits was estimated as a free parameter in the model. After this correlation was estimated the hypotheses that there were additional, significant associations between specific brain regions and cognitive functions was tested. Ninety-three subjects had complete data for the cognitive tests and MRI variables shown in Figure 4.2. The statistical ‘fit’ of the model in Figure 4.2 is described first, and then the meaning of the model is explained.

There are several different indications of fit adequacy in EQS. The average of the off-diagonal standardized residuals was 0.047, indicating that most of the covariance was accounted for by the model. The largest two residuals were between temporal lobe volume and AVLT and Logical Memory scores, and were *negative* in direction. The chi square for the model was 35.6 (df=41, p=0.71). Non-significant chi-square values indicate good fit. EQS gives three fit indices which have values between 0 and 1, with values over 0.9 indicating good fit. For the model in Figure 4.2 these were: Bentler-Bonett normal fit index = 0.91; Bentler-Bonett non-normal fit index = 1; comparative fit index = 1.0. All indicated a well-fitting model. All of the pathways in the model were significant and the Wald test indicated that no pathways in the model should be dropped.

The model in Figure 4.2 shows that individual variation in the three brain areas was substantially derived from overall brain size, here derived as a latent trait.

Figure 4.2: Structural equation model of the relationships among individual brain region volumes, the general brain size factor, and the individual cognitive tests and the general cognitive factor.



Abbreviations: **NART:** National Adult Reading Test; **RSPM:** Raven's Standard Progressive Matrices; **DSST:** Digit-Symbol Substitution Test; **BVRT:** Benton Visual Retention Test; **VR:** Visual Reproduction; **AVLT:** Rey Auditory-Verbal Learning Test; **LM:** Logical Memory; **VF:** verbal fluency. All relationships are at $p < 0.05$.

The covariance among the cognitive tests was successfully captured by hypothesising that this was derived substantially but not wholly from a general cognitive latent trait. These two latent traits - 'overall brain size' and 'general cognitive function' - correlated 0.42. Note that two verbal tests (National Adult Reading Test and Verbal Fluency) and two verbal memory tests (Auditory-Verbal Learning Test and Logical Memory) had correlated residuals. This reflects the similarity of the two pairs of tests, which are indicators of 'group factors' of ability. The Lagrange Multiplier Test showed that there were no additional significant paths between specific cognitive tests and brain size or between specific brain region volumes and the general cognitive factor.

4.4 Discussion

The main findings were that in this sample of 97 healthy men aged 65-70, intracranial area correlated positively with tests of general intelligence and visuospatial memory. Positive correlations between these tests and several regional brain volumes were also found. When regional brain volumes were adjusted for ICA there were virtually no significant correlations between brain volumes and cognitive tests.

There were significant positive correlations among all the cognitive tests, suggesting much shared variance among the tests. This was confirmed by principal components analysis, in which the first unrotated component accounted for 50% of the total variance. Thus these findings replicate the vast majority of previous studies which have examined correlations in performance on diverse cognitive tests (Carroll, 1993).

A key finding in this study was the set of significant positive correlations between several cognitive tests and ICA. In studies of cognitive ageing which use structural brain imaging, brain volumes are often adjusted for a measure of head size.

However, these findings indicate that the present measure of head size, ICA, may itself be of some importance. In fact, in this relatively large dataset adjusting for ICA removed virtually all of the associations. These findings have implications for research into brain structure-cognitive function relations, particularly in the field of mild cognitive impairment, where deficits in cognitive function and changes in the brain are subtle. Many studies report an association between hippocampal size and deficits in declarative memory in mild cognitive impairment and in Alzheimer's disease (Jack et al., 1997), but using sensitive tests and detailed imaging in a large sample of healthy elderly men this study did not demonstrate such an association. This concurs with a recent study which found a *negative* association between hippocampal volume and performance on a delayed memory task in 75 women aged 18-30 (Foster et al., 1999). Although there is often severe hippocampal atrophy in frank Alzheimer's disease which is associated with deficits in declarative memory (Petersen et al., 2000), the present findings suggest that specific changes in

hippocampal volume may only become an important variable in the presence of pathology.

Smaller head size has been linked with lower cognitive ability in a large sample of non-demented elderly adults (Reynolds et al., 1999) and may also be a risk factor for Alzheimer's disease (Graves et al., 1996). These findings have been advanced in the context of the 'cognitive reserve hypothesis' which proposes that individuals with larger brains can sustain more relatively more damage from a pathological process before developing the overt symptoms of dementia (however there are some studies which have failed to show this (Jenkins et al., 2000)). However, there are other possible explanations. For example, it is well established that low birth weight is linked with the insulin resistance syndrome (Seckl, 1997), and hyperinsulinaemia is a risk factor for cognitive impairment in later life (Vanhanen et al., 1998). Small head size clusters with low birth weight and therefore associations between head size and cognitive function in late life may be partially mediated by metabolic factors. Indeed, small head circumference at birth has been shown to associate with an increased risk of cardiovascular mortality at age 65 (Barker et al., 1993), suggesting that other vascular disease (ie. cerebrovascular) may also cluster with small head size as determined by early life events.

Using structural equation modelling to best describe the underlying relationships among the cognitive tests and brain volumes it was found that the best-fitting model suggested that the relationship between overall cognitive ability and a common factor representing hippocampi, temporal lobes and frontal lobes best accounts for the data. That is, relationships between performance on specific cognitive tests and specific brain regions are best accounted for by these variables' respective contributions to the general cognitive and brain volume factors. In summary, in terms of brain volumes, the main association was between *overall* brain size and *general* cognitive ability.

There are certain methodological limitations of the study. Whole brain, parietal, occipital or cerebellar volumes were not measured and specific associations between these regions and cognitive tests cannot be ruled out. The sample was all men and

thus the findings cannot be confidently generalized to women. The sample can be described as “young-elderly” and was selected for good health and thus any effects of ageing on cognitive function and on brain volumes are likely to be slight; the present findings may have more in common with studies looking at younger populations. However, it is possible some subjects were in subclinical stages of dementia, which could affect both brain volumes and cognitive function. Only a prospective study commencing in mid-life or earlier could examine this question adequately.

Chapter 5: Cognitive function and magnetic resonance spectroscopy

5.1 Introduction

Deficits in cognitive function become more common with ageing. Alzheimer's disease and other dementias are at the severe end of the spectrum, but milder deficits in cognitive function are also important in terms of the associated morbidity and also because milder deficits are a risk factor for the development of dementia (National Research Council, 2000). The mechanisms underlying these changes are poorly understood. Pathological studies demonstrate that neuronal loss does occur in dementias such as Alzheimer's disease but there may be very little neuronal loss in the absence of such disease processes (West et al., 1994) suggesting that changes in the function of neurons might play an important role in cognitive ageing.

As discussed in Chapter 1, N-acetylaspartate (NAA) is thought to be specific to neurons (Simmons et al., 1991) and some studies have suggested that NAA levels are reduced in conditions in which there is known to be neuronal loss, such as Alzheimer's disease (Schuff et al., 1998). NAA levels are therefore often used as a marker of 'neuronal integrity'. Choline levels reflect the quantities of free choline-containing compounds, which are involved in both the synthesis and breakdown of phospholipid membranes (Pettegrew et al., 1997). Creatine levels are thought to reflect the quantity of phosphorylcreatine and creatine, involved in energy metabolism, in neurons and glial cells (Pettegrew et al., 1995; Wyss and Kaddurah-Daouk, 2000). However, whether there are variations in levels of NAA or other metabolites such as choline (Cho) and creatine (Cr) in milder ageing-related cognitive impairments, in which changes in the brain might be more subtle, is unknown.

In this study, relationships between tests of cognitive function and metabolite levels using H1-MRS in a large sample of healthy elderly men are explored. The main hypothesis was that higher levels of NAA as estimated by NAA/Cr and NAA/Cho ratios correlate with better cognitive performance.

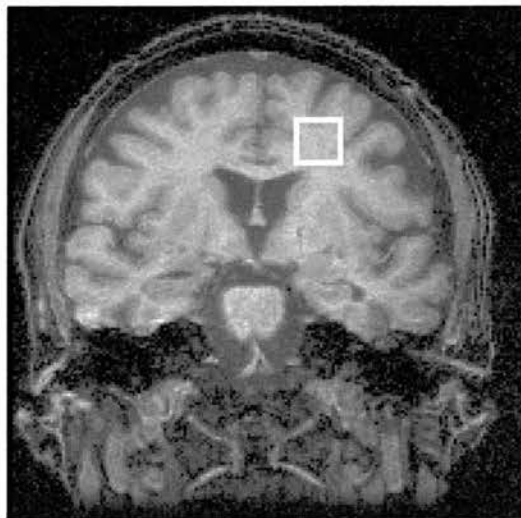
5.2 Methods

Subjects and cognitive testing were as described in Chapter 2.

5.2.1 Neuroimaging

Spectroscopic analyses were conducted by Dr. Karen J Ferguson, Professor Joanna M Wardlaw and Dr. Ian Marshall (Appendix 1). Placement of the voxel is shown in Figure 5.1. These analyses yielded data for NAA, Cho and Cr levels. These metabolite levels are in ‘institutional units’, that is, they are not directly related to absolute concentrations. Raw data are not comparable between subjects and thus metabolite levels are traditionally analysed as ratios.

Figure 5.1: Placement of the voxel⁴ in the parietal cortex



⁴ The term “voxel” refers to the element of 3-D space corresponding to a pixel, for a given slice thickness.

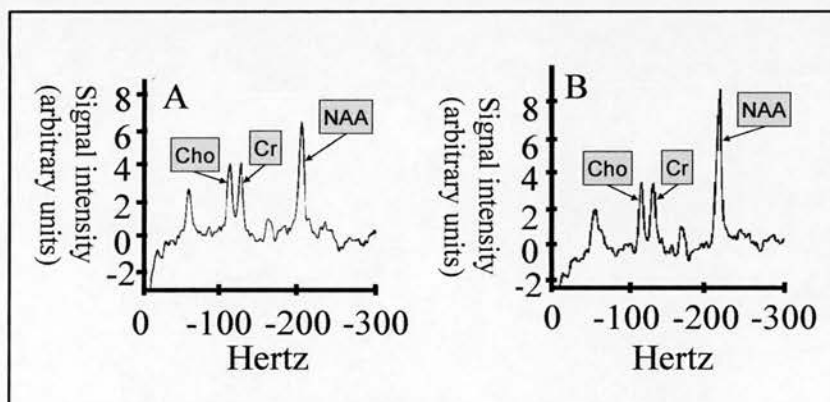
5.2.2 Statistical analysis

To avoid making assumptions about whether any particular metabolite levels remained relatively constant, an alternative method to the use of metabolite ratios was developed by the author and Professor Ian Deary. In this method, each metabolite value was regressed against the remaining two and the standardised residual values saved. This method is similar in aim to the use of ratios but has the advantage of not assuming that creatine levels are an appropriate internal control. In the present chapter these derived values are termed “adjusted metabolite levels”. Because of the substantial positive intercorrelations among cognitive test scores (as discussed in Chapter 1) (Carroll, 1993), the cognitive test data were subjected to principal components analysis (PCA), excluding the National Adult Reading Test because it is a measure of premorbid or prior IQ, and Logical Memory 24-hr delayed because the number of subjects was substantially less than for the other tests. Individual cognitive test results and factor scores derived from PCA were correlated with metabolite ratios and with the adjusted metabolite levels derived as described above.

5.3 Results

Of the 100 participants scanned, three were excluded when unexpected pathology was discovered on structural MRI: two because of congenital arachnoid cysts, and one because of a pituitary adenoma (non-secretory). Of the remaining 97 subjects, 88 spectra were suitable for metabolite quantification analysis as determined by evaluation of the appearance of the spectra by a neuroradiologist (Professor JM Wardlaw) and assessment of the quality of curve fitting during analysis of spectra by a research fellow (Dr. KJ Ferguson). Data from two further spectra were excluded as the standardised residual values in the linear regression analysis were greater than three standard deviations from the mean. Sample spectra from the present study are shown in figure 5.2.

Figure 5.2: Sample spectra from magnetic resonance spectroscopy



These spectra show high (the spectrum on the left) and low (the spectrum on the right) Cho and Cr in relation to NAA.

Principal components analysis of the cognitive tests revealed two factors with eigenvalues greater than one, explaining a total of 66% of the variance. The factors were subjected to Direct Oblimin rotation. Because of the pattern of loadings (as described in Chapter 3), the two factors were designated the Visuospatial / Fluid

Factor and the Verbal Memory Factor. These factor loadings were entered into the correlational analysis in addition to the raw cognitive test scores with the metabolite ratios and the adjusted metabolite levels.

For the correlational analyses, there were 86 subjects for all comparisons except: Raven's Standard Progressive Matrices (N=84), where two subjects were excluded because of incorrect completion of the answer sheets; Logical Memory (N=85), where one subject's score was invalid in this test because of interruption of the testing session, and Visual Reproduction (N=85); where a further subject also had the test session interrupted. Listwise deletion meant that factor scores from 82 subjects were available. For Logical Memory 24-hr delayed 58 subjects returned for retesting, of which 50 had spectra suitable for inclusion.

Correlations between metabolite level ratios and cognitive test results are shown in Table 5.1. NAA/Cr ratios were positively correlated with Logical Memory ($r=0.25$, $p<0.05$), Logical Memory 24-hr delayed $r=0.43$, $p<0.01$) and the Verbal Memory Factor ($r=0.25$, $p<0.05$). Cho/Cr ratios were positively correlated with the Benton Visual Retention Test, Visual Reproduction, the Auditory-Verbal Learning Test, Logical Memory, Logical Memory 24-hr delayed, and the Verbal Memory Factor (r values 0.21 to 0.38, $p<0.05$). NAA/Cho ratios did not correlate significantly with any test scores.

Table 5.1: Pearson correlations between metabolite ratios and cognitive test results

	NAA/Cho	NAA/Cr	Cho/Cr
National Adult Reading Test	-.17	.13	.20
Raven's Matrices	-.00	.19	.15
Benton Visual Retention Test	-.15	.17	.25*
Visual Reproduction	-.05	.21	.21*
Digit-Symbol Substitution Test	-.06	.06	.07
Auditory-Verbal Learning Test	-.08	.21	.22*
Logical Memory	-.10	.24*	.25*
Logical Memory (24-hr delayed)	-.07	.43**	.38**
Verbal Fluency	-.18	-.01	.11
Visuospatial / Fluid Factor	-.09	.16	.18
Verbal Memory Factor	-.09	.25*	.25*

*p<.05 (2-tailed) ** p<.010 (2-tailed).

N=86 for all tests except Raven's Standard Progressive Matrices (N=84), Logical Memory (N=85), Visual Reproduction (N=85) and Logical Memory – 24hr-delayed (N=50) and the Visuospatial / Fluid Factor and Verbal Memory Factors (N=82).

The correlations between the adjusted metabolite levels and cognitive test results are shown in Table 5.2. Adjusted creatine levels were significantly negatively correlated with Logical Memory ($r=-0.26$, $p<0.05$), Logical Memory 24-hr delayed ($r=-0.39$, $p<0.01$) and the Verbal Memory Factor ($r=-0.26$, $p<0.05$). There were trends in the same negative direction with Raven's Standard Progressive Matrices, Visual

Reproduction, and the Auditory-Verbal Learning Test. Adjusted choline levels and adjusted NAA levels did not correlate significantly with any of the cognitive test scores.

Table 5.2: Pearson correlations between adjusted metabolite levels and cognitive test results.

	Adj_Cr	Adj_Cho	Adj_NAA
National Adult Reading Test	-.20	-.19	-.08
Raven's Matrices	-.18	.03	.10
Benton Visual Retention Test	-.12	.20	.05
Visual Reproduction	-.17	.13	.21
Digit-Symbol Substitution Test	-.05	.07	.06
Auditory-Verbal Learning Test	-.19	.12	.08
Logical Memory	-.26*	.14	.05
Logical Memory (24-hr delayed)	-.39**	.13	.17
Verbal Fluency	-.01	.21	-.11
Visuospatial / Fluid Factor	-.11	.15	.11
Verbal Memory Factor	-.26*	.13	.09

**p<.01 (2-tailed) *p<.05 (2-tailed)

N=86 for all tests except Raven's Standard Progressive Matrices (N=84), Logical Memory (N=85), Visual Reproduction (N=85) and Logical Memory – 24hr-delayed (N=50) and the Visuospatial / Fluid Factor and Verbal Memory Factors (N=82).

Abbreviations: Adj_Cr: adjusted creatine; Adj_Cho: adjusted choline; Adj_NAA: adjusted NAA.

5.4 Discussion

The main findings in this study were that higher NAA/Cr and Cho/Cr ratios were associated with better performance on several cognitive tests. Both ratios correlated positively with Logical Memory, Logical Memory 24-hr delayed and a derived Verbal Memory Factor. Cho/Cr also correlated positively with the Benton Visual Retention Test, Visual Reproduction and the Auditory–Verbal Memory Test. Higher adjusted creatine levels were associated with worse performance on Logical Memory, Logical Memory 24-hr delayed and the Verbal Memory Factor. All of the remaining cognitive variables correlated in a negative direction with adjusted creatine and the strength of many of these correlations approached statistical significance. There were no correlations between NAA/Cho ratios, adjusted NAA levels or adjusted choline levels and cognitive tests or the derived factors.

This is the largest study to date examining brain metabolite-cognition associations in a healthy human sample and is the first to show these relationships. The findings have implications for the understanding of the mechanisms underlying the variations in cognitive ageing. However, the findings also raise important issues concerning the use of metabolite ratios instead of absolute values. Most ¹H-MRS studies do not use water-referencing or phantom-referencing for the calculation of metabolite levels and thus express results as ratios. Traditionally, creatine levels are thought to show less inter-individual variation (Catani et al., 2001) and thus are commonly used as the denominator. They are also considered to change little in disease, or only because of marked disease. The practice of using ratios may also be because of the emphasis placed on NAA as a marker of neuronal integrity. Some studies of normal ageing have reported reductions in NAA/Cr (Angelie et al., 2001). In most published papers reductions in NAA/Cr are interpreted as signifying reductions in absolute NAA, but, clearly, increases in creatine would have the same arithmetical effect, and perhaps the previous studies should be reanalysed in this light. Using the traditional method of analysis, the present findings would suggest that higher NAA and choline levels are associated with better cognitive function. However, the regression analysis

suggests that higher creatine levels might better explain the correlations between NAA/Cr and Cho/Cr ratios and cognitive variables rather than lower NAA or choline levels. Alternatively, the correlations with adjusted creatine might reflect the association of reduced levels of *both* NAA and choline levels with cognitive variables. Only measurement of the absolute levels of these metabolites would resolve these alternatives.

There is some evidence that creatine levels may themselves be important in the pathophysiology of cognitive ageing. Some H1-MRS studies using absolute metabolite concentrations have reported higher creatine levels in healthy ageing brains compared with healthy younger brains (Pfefferbaum et al., 1999b; Saunders et al., 1999; Leary et al., 2000; Schuff et al., 2001). Creatine levels as detected by H1-MRS reflect the sum of phosphocreatine (PCr) and creatine. PCr is present in the brain at high levels, providing a store of ~P for phosphorylation of ADP and thus maintaining ATP levels. The conversion of phosphocreatine to ATP is catalyzed by creatine kinase. Creatine kinase levels, activity and forward flux decrease in ageing (Smith et al., 1991; Smith et al., 1997) while P31-MRS studies have shown increasing PCr levels in the ageing rat (Pettegrew et al., 1990). Pettegrew and co-workers (Pettegrew et al., 1995) identified a cognitively normal man of 55 who had P31-MRS changes suggestive of dementia. Follow-up over 46 months showed a progressive decline in cognitive function to frank dementia, paralleled closely by a linear rise in PCr. Control subjects showed no changes in PCr. Although the number of studies and their samples sizes are small, together these findings suggest the possibility that the conversion of phosphocreatine to ATP may be impaired in ageing, accounting for an accumulation of PCr. Thus, increased levels of creatine may be a marker of decreased brain energy metabolism (Wyss and Kaddurah-Daouk, 2000) which may be associated with ageing-related mild cognitive impairment, as well as, in more extreme cases, frank dementia.

This study did not find any relationship between adjusted NAA and cognitive function. This may reflect the healthy state of the participants since NAA is thought to be a marker of neuronal number and integrity. Other studies, with subject numbers

of between 30 and 50, have reported that NAA is not reduced in the white matter of healthy elderly subjects (Pfefferbaum et al., 1999b; Lundbom et al., 1999; Saunders et al., 1999) although one study did find decreased NAA in 50 healthy elderly subjects (Brooks et al., 2001). Broadly speaking, however, these findings fit with stereological findings suggesting that brain atrophy in healthy ageing is not caused by neuronal death (West et al., 1994).

Adjusted choline levels did not correlate significantly with any cognitive variables. Interpretation of choline levels derived from H1-MRS is difficult as free choline is increased both in membrane breakdown (eg. necrosis) and increased formation (eg. gliomas). This confusion is reflected in the variety of findings for choline changes with ageing: higher choline in grey matter (Pfefferbaum et al., 1999b; Pfefferbaum et al., 1999a; Chang et al., 1996), lower choline in grey matter (Charles et al., 1994) and no change in choline levels in white matter with ageing (Pfefferbaum et al., 1999b; Saunders et al., 1999; Schuff et al., 2001; Schuff et al., 1999; Lundbom et al., 1999) have all been reported.

There are several methodological limitations of the present study which should be noted. Without determining absolute concentrations of metabolites, interpretation of the results must remain speculative. Regression analysis was used in an attempt indirectly to look for the unique variance for each metabolite, but, as with ratios, the biological significance of metabolite values generated with this method is unclear. Techniques which generate absolute values are extremely time-consuming and, especially with older people, the long scanning times make large-scale studies impractical. Even in techniques which generate values to be used in ratios, H1-MRS is not free of problems, in that subjects must lie still for considerable periods; in large groups it is likely that a proportion of subjects will not tolerate these long scanning times. Even in co-operative subjects, there may be problems with the technique, like poor shimming (this procedure is carried out to optimise the homogeneity of the magnetic field around the subject), which preclude obtaining measurable spectra. The study is cross-sectional, so it cannot be known whether individual differences in

metabolite levels are longstanding at this stage in life or represent differences accruing with brain ageing which may predate overt dementia

In conclusion, this study provides evidence that individual differences in metabolite levels relate significantly to variations in cognitive function at age 65 to 70. The pattern of results seen in the ratios, as well as the regression analysis, suggest that creatine but not choline and NAA are associated with these variations. This is in contrast to studies focusing on neurodegenerative diseases, which suggest that lower NAA levels are associated with cognitive decrements. Further H1-MRS studies using spectroscopy protocols which can generate absolute levels of these metabolites are essential to clarify the nature of these associations. The use of P31-MRS may also help to understand the relative concentrations of PCr and creatine in ageing brain and to examine further the hypothesis that individual differences in cognitive ageing may stem in part from changes in brain energy metabolism.

Chapter 6: Cortisol levels, and neuroimaging and cognitive function

6.1 Introduction

The hypothesis that a proportion of ageing animals and humans develop rising levels of glucocorticoids and that these elevations are linked with atrophy of certain structures of the brain and cognitive decrements was first proposed many decades ago and received its first empirical support in the work of Landfield (Landfield et al., 1978; Landfield, 1981). Sapolsky developed this notion with the glucocorticoid cascade hypothesis, in which reduced feedback sensitivity to glucocorticoid levels at the level of the hippocampus leads to higher glucocorticoid levels which are damaging to the hippocampus, leading to further reductions in feedback sensitivity, and so on (Sapolsky et al., 1986).

Currently the direct evidence in favour of this hypothesis is mixed. It is clear that the hippocampus is involved in feedback regulation of the hypothalamic-pituitary-adrenal (HPA) axis and under most conditions exerts an inhibitory effect (Seckl and Olsson, 1995). In animals, experimental lesions to the hippocampus cause rises in glucocorticoids. In humans the effects of hippocampal lesions on feedback regulation of the HPA axis are less clear. In diseases in which there is damage to the hippocampus, such as Alzheimer's disease, there is evidence of disturbance in feedback regulation of the HPA axis. Some studies have shown reduced suppression of cortisol following dexamethasone (Murialdo et al., 2000) and others have shown increased overall output of glucocorticoids (Rasmuson et al., 2001). However, it is not possible to be certain that these disturbances are all due to compromise of the role of the hippocampus in HPA axis regulation, as Alzheimer's disease involves widespread damage to the brain, and there are other routes by which glucocorticoids might rise, such as with the depressive illness commonly seen in Alzheimer's disease. What is clearer is that there is increased variability in the feedback

sensitivity of the HPA axis with ageing, with older animals and humans more often showing a slower decline in glucocorticoid levels after a stressful episode (Seeman and Robbins, 1994; Seckl and Olsson, 1995), or showing reduced sensitivity to suppression by dexamethasone (Ferrari et al., 2001).

Regarding elevations in glucocorticoids with ageing, animal and human studies, though few in number, do suggest that there is an increase in variability in overall glucocorticoid secretion. An even smaller number of studies has linked these elevations with decrements in cognitive function (Issa et al., 1990; Lupien et al., 1996; Seeman et al., 1997). Some studies have suggested that interventions such as adrenalectomy and low-dose corticosterone replacement (Landfield, 1981), and administration of antidepressants from mid-life (which is associated with lower corticosterone levels in ageing animals) (Yau et al., 2002), are associated with an attenuation of ageing-related cognitive decrements.

Neuroimaging studies linking cognitive function, hippocampal volumes and indices of HPA axis activity are few. Most of these studies involve patients with Cushing's syndrome or Alzheimer's disease. The studies in Cushing's syndrome suggest that cognitive function is affected across several domains; in terms of neuroimaging studies using modern techniques have only examined the possibility that there is hippocampal atrophy; no other regions have been studied (Starkman et al., 2001; Starkman et al., 1992). The only study looking at the whole brain used pneumoencephalopathy and found global atrophy in patients with Cushing's syndrome versus controls patients who had acromegaly (Momose et al., 1971). In Alzheimer's disease a higher degree of hippocampal atrophy has been linked with hypercortisolaemia (deLeon et al., 1988; Magri et al., 2000). There are very few studies of this type in older healthy adults. Lupien and co-workers (Lupien et al., 1998) examined cortisol levels over five years in 11 healthy elderly subjects and then measured cognitive function and hippocampal, parahippocampal gyrus, fusiform gyrus, superior temporal gyrus, and middle and inferior temporal gyri volumes, controlled for head size. They divided the subjects into two groups based on the pattern of change in cortisol secretion over the five years: an 'increasing/high' group

and a 'decreasing/moderate' group. They found that the 'increasing/high' group had 14% smaller hippocampi (4.00cm³ versus 4.54cm³, $p < 0.001$) and significantly poorer performance on the delayed declarative memory task. There were no significant differences for the other brain volumes. Although interesting, the number of subjects is too small to draw any strong conclusions from this study. In a cross-sectional study, Wolf and co-workers (Wolf et al., 2002) did not find a correlation between hippocampal volume and cortisol output in 11 men aged 59-76; cognitive function was not measured. Thus there are no adequately-sized studies which have examined the links among volumetric neuroimaging variables, in particular hippocampal volume, in normal ageing populations. There are no published studies examining links among glucocorticoid measures and other types of neuroimaging variables, such as white matter lesions or metabolite levels as measured by magnetic resonance spectroscopy.

In this chapter data gathered from glucocorticoid measurements are presented and analysed. Then the hypotheses that glucocorticoid levels are related to cognitive function and also to brain region sizes are examined. Glucocorticoid levels are also analysed in relation to metabolite levels as measured by magnetic resonance spectroscopy.

6.2 Methods

Three domains of measurements are used: neuroimaging, glucocorticoids and cognitive function. Neuroimaging was performed using structural magnetic resonance imaging, giving data on regional brain volumes, intracranial capacity, and magnetic resonance spectroscopy (Appendix 1). 9am, 2.30pm and post-dexamethasone (0.25mg) plasma cortisol levels were obtained as detailed in Chapter 2. The cognitive testing battery was also described in Chapter 2.

Radioimmunoassay

10ml of blood was taken and immediately spun at 3000rpm at 4°C. Then 2-3ml of plasma was extracted and stored at -20°C until analysis. 10µl aliquots of plasma from each sample were diluted with 290µl of the assay diluent (100nM of citrate-phosphate buffer at pH 4.0 containing 0.1% gelatin). A pre-prepared set of standards (concentrations: 10,20,40,80 ... 5120nM) was also used. Samples and standards were prepared in triplicate in disposable LP4 (Luckhams) polystyrene tubes. Solutions were mixed with 50µl 125I-cortisol (producing around 10000 counts per minute) and 50µl cortisol antibody (SAPU: Scottish Antibody Production Unit). The tubes were vortexed gently and incubated at room temperature for 4 hours. Then 50µl of the second antibody (donkey anti-sheep/goat serum @ 1:15 initial; SAPU) and 50µl non-immune sheep serum (@1:200 initial with 1nM EDTA; SAPU) were added. The tubes were incubated at 4°C overnight and then spun at 4°C for 45 minutes at 1720 G. The supernatant was decanted to waste and the bound fraction counted in a gamma-counter (LKB Wallac 1260).

Samples were rejected if there was a greater than 10% difference (of the smaller value) between the counts. The mean intraassay coefficient of variation was 6.2%. The interassay coefficient of variation (calculated through quality control samples included in each batch) was 9.2%.

Statistical Analyses

Neuroimaging and cognitive data were explored and prepared as detailed in earlier chapters. Cortisol levels from the three types of measurement: 9am, 2.30pm and post-dexamethasone levels were first subjected to data exploration. Relationships among these levels were then examined with correlations and principal components analysis. Neuroimaging and cognitive data were then examined in turn in relation to the cortisol levels using correlations and principal components analyses.

6.3 Results

Cortisol levels: descriptive data

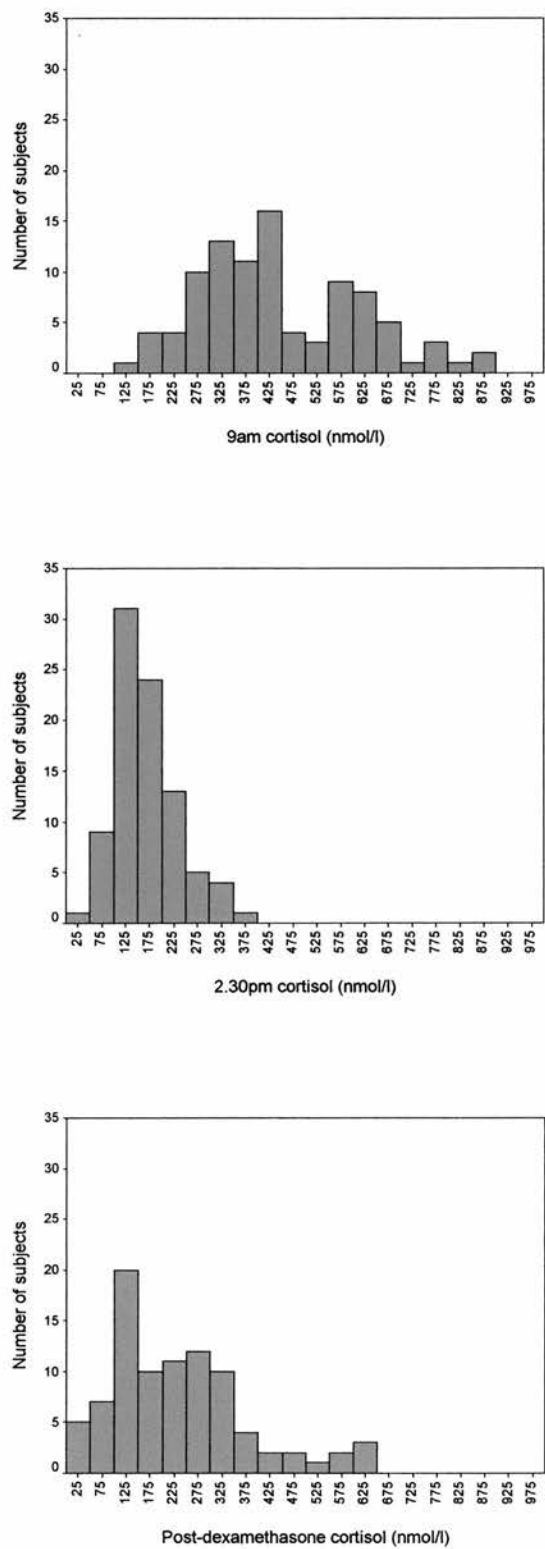
Descriptive data for 9am, 2.30pm and post-dexamethasone cortisol are shown in Table 6.1. Of the 97 possible 9am cortisol levels, two were unavailable for reasons of technical failure. Not all subjects were able to return for testing of 2.30pm cortisol levels or for testing of post-dexamethasone cortisol levels, hence the N's of 88 and 89, respectively.

As expected, 9am cortisol levels were the highest of the three cortisol measures. 2.30pm levels were the lowest and post-dexamethasone levels were intermediate. Histograms for 9am, 2.30pm and post-dexamethasone levels are shown in Figure 6.1. These show that 2.30pm and post-dexamethasone cortisol levels were somewhat skewed to the left, whereas the 9am levels showed a normal distribution. The distributions were not so skewed as to preclude analysis with parametric tests. However, scatterplots for each comparison were examined.

Table 6.1: Cortisol levels: descriptive data

	N	Minimum	Maximum	Mean	SD
9am cortisol (nmol/l)	95	105	898	444.1	171.0
2.30pm cortisol (nmol/l)	88	23	369	168.1	66.0
Post-dex cortisol (nmol/l)	89	38	632	233.5	142.0

Figure 6.1: Histograms of 9am, 2.30pm and post-dexamethasone cortisol levels



Cortisol levels showed positive intercorrelations, with 9am cortisol correlating at $r=0.26$ ($p=0.017$, $N=86$) with 2.30pm cortisol and at $r=0.24$ with post-dexamethasone cortisol ($p=0.001$, $N=88$). A correlation matrix for the Spearman's correlations is provided in Appendix 3. These correlations are similar. There was a non-significant positive correlation between 2.30pm cortisol and post-dexamethasone cortisol ($r=0.17$, $p=0.108$, $N=84$).

Table 6.2: Pearson correlations among cortisol measures

	2.30pm cortisol	Post-dex cortisol
9am cortisol	.26 ($p=0.017$, $N=86$)	.34 ($p=0.001$, $N=88$)
2.30pm cortisol		.17 ($p=0.108$, $N=84$)

Cortisol levels and brain volumes

Each of the cortisol measures was correlated against brain volumes. These showed some small, significant, negative correlations with the cortisol measures. Non-significant correlations tended also to be negative. Scatterplots were then performed for each correlation. Because these revealed the possibility that outliers might affect the correlations (sample scatterplots are shown in Figure 6.2), correlations were repeated using the nonparametric equivalent of Pearson's r , Spearman's ρ . Both Pearson and Spearman correlations are shown in Table 6.3. The nonparametric correlations show that the left temporal lobe correlated negatively and significantly with 9am cortisol ($r=-0.22$) and 2.30pm cortisol ($r=-0.26$), the right temporal lobe correlated with 9am cortisol ($r=-0.21$), the right hippocampus correlated with 9am cortisol ($r=-0.22$) and post-dexamethasone cortisol ($r=-0.24$). These correlations were

significant at $p < 0.05$, 2-tailed. The left hippocampus showed no significant correlations but showed a trend towards a negative correlation with 9am cortisol ($r = -0.19$, $p = 0.065$) and the left frontal lobe showed a trend towards a negative correlation with 2.30pm cortisol ($r = -0.21$, $p = 0.052$). Brain volumes adjusted for intracranial area showed correlations in the same negative direction, but all but one (2.30pm cortisol and left temporal lobe $r = 0.025$ ($p = 0.018$)) were statistically nonsignificant.

Figure 6.2: Sample scatterplots hippocampal volumes and cortisol levels

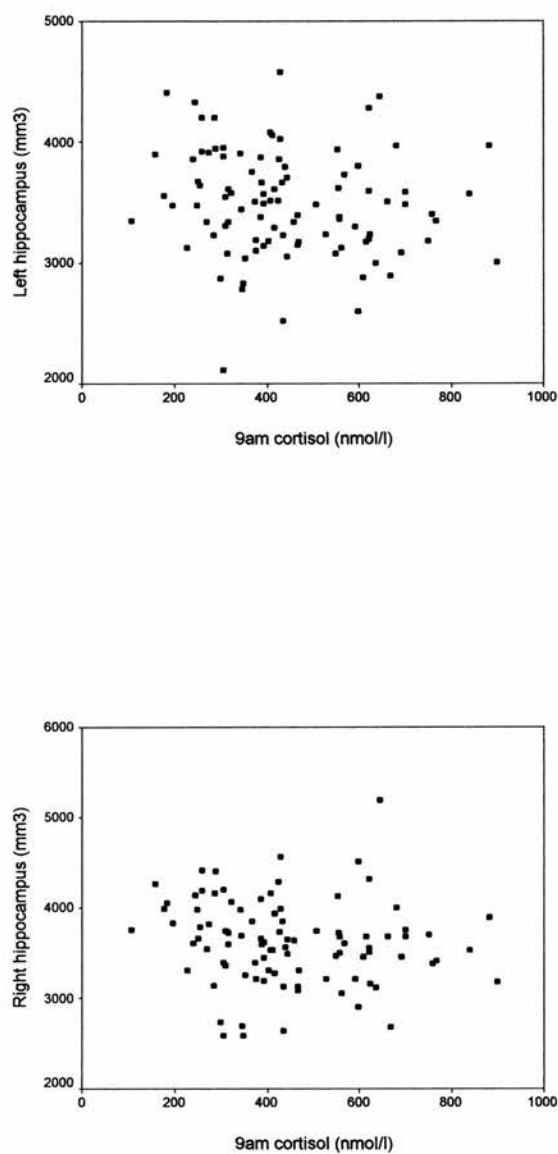


Table 6.3: Pearson and Spearman correlations between brain volumes and cortisol measures

	Type of correlation	9am cortisol	2.30pm cortisol	Post-dex cortisol
Left hippocampus	<i>Pearson</i>	-.015	-.03	-.16
	<i>Spearman</i>	-.19 (p=0.065)	-.08	-.16
Right hippocampus	<i>Pearson</i>	-.12	-.02	-.25* (p=0.018)
	<i>Spearman</i>	-.22* (p=0.034)	-.04	-.24* (p=0.021)
Left frontal lobe	<i>Pearson</i>	.02	-.22* (p=0.044)	-.03
	<i>Spearman</i>	-.02	-.21 (p=0.052)	-.02
Right frontal lobe	<i>Pearson</i>	.03	.01	-.15
	<i>Spearman</i>	-.02	.02	-.13
Left temporal lobe	<i>Pearson</i>	-.21* (p=0.038)	-.20	-.04
	<i>Spearman</i>	-.22* (p=0.033)	-.26 (p=0.016)	0
Right temporal lobe	<i>Pearson</i>	-.16	.02	-.15
	<i>Spearman</i>	-.21* (p=0.038)	-.03	-.12

N=95 for 9am cortisol correlations, N=88 for 2.30pm cortisol correlations and N=89 for post-dexamethasone cortisol correlations.

Cortisol levels and magnetic resonance spectroscopy

Cortisol levels were correlated against metabolite ratios and adjusted metabolite levels using the Pearson correlation coefficient. The correlations are shown in Table 6.4. None of the correlations was significant.

Table 6.4: Correlations between cortisol levels, and metabolite ratios and adjusted metabolite values

	9am cortisol	2.30pm cortisol	Post-dex cortisol
NAA / Cho	.07	-.04	-.01
NAA / Cr	-.03	-.04	-.19
Cho / Cr	-.07	.02	-.16
Adj_NAA	.06	-.12	-.11
Adj_Cho	-.11	.01	-.06
Adj_Cr	.01	-.02	.13

Cortisol levels and cognitive function

Cortisol measures were correlated against cognitive test scores. The correlations are presented in Table 6.5. 9am cortisol correlated with Logical Memory 24 hour delayed correlated at $r=-0.31$ ($p=0.018$, $N=58$) and Raven's Standard Progressive Matrices at $r=-0.21$ ($p=0.044$, $N=93$). Of the remaining correlations, several showed non-significant trends towards correlations in the predicted direction.

Table 6.5: Pearson correlations between cortisol levels and cognitive tests

	9am cortisol	2.30pm cortisol	Post-dex cortisol
NART	.01	-.05	.06
Verbal Fluency	-.08	-.04	-.02
DSST	-.17	-.15	-.07
AVLT	-.07	-.20	-.03
Logical Memory	-.11	.10	-.01
Logical Memory 24hr	-.31* ($p=0.018$)	-.17	-.09
BVRT	-.11	-.10	-.06
Visual Reproduction	-.12	-.04	.03
RSPM	-.21* ($p=0.044$)	-.14	-.11

For Logical Memory 24 hr delayed, $N=58$ for 9am cortisol levels, $N=55$ for 2.30pm cortisol levels, and $N=54$ for post-dexamethasone levels. For all other tests, 9am cortisol levels, N 's=93 to 95; 2.30pm cortisol levels N 's=86 to 88; post-dexamethasone cortisol levels, N 's 87 to 89.

Abbreviations: **NART:** National Adult Reading Test; **DSST:** Digit-Symbol Substitution Test; **AVLT:** Auditory-Verbal Learning Test; **BVRT:** Benton Visual Retention Test

Data reduction with principal components analysis (yielding first a general cognitive factor and then, following Direct Oblimin rotation, two correlated factors, the visuospatial / fluid factor and the verbal memory factor) was carried out as described in Chapter 5. There were no significant correlations between cortisol measures and the cognitive factors.

Cognitive tests were then adjusted for premorbid IQ (using linear regression). The purpose of this was to control for the influence of baseline general cognitive ability. The adjusted cognitive test scores were then correlated against the cortisol measures. The pattern of correlations was similar to the correlations with the unadjusted tests, with all but two correlations being in the negative direction but only two reaching statistical significance. These were both with 9am cortisol, with the Digit-Symbol Substitution Test correlating at $r=-0.21$ ($N=95$, $p=0.04$) and Logical Memory 24h delayed correlating at $r=-0.28$ ($N=58$, $p=0.03$).

Because there were several negative correlations between the adjusted cognitive test scores and the cortisol measures, the cognitive factors were also adjusted for premorbid IQ and correlated against the cortisol measures. Both the adjusted general cognitive factor and the adjusted visuospatial / fluid factor correlated with 9am cortisol at $r=-0.23$ ($N=91$, $p=0.028$). There were no significant correlations with the other cortisol measures, though all correlations were in the predicted direction (Table 6.7).

Table 6.7: Correlations between cortisol levels and adjusted cognitive factors

	9am cortisol	2.30pm cortisol	Post-dex cortisol
General cognitive factor (adjusted)	-.23* (p=0.028)	-.15	-.11
Visuospatial / fluid factor (adjusted)	-.23* (p=0.028)	-.12	-.12
Verbal memory factor (adjusted)	-.10	-.15	-.04

N=91 for 9am cortisol levels, N=84 for 2.30pm cortisol levels and N=85 for post-dexamethasone cortisol levels.

6.4 Discussion

In this chapter, three different cortisol measures were correlated against brain volumes and metabolite levels as measured by magnetic resonance spectroscopy. The cortisol measures were, 9am levels, 2.30pm cortisol and post-dexamethasone. These cortisol levels were also correlated against cognitive function. The main hypotheses, given the previous animal and human work, were that cortisol levels would correlate negatively with hippocampal volumes, and that cortisol levels would correlate negatively with cognitive function.

9am cortisol levels correlated significantly and negatively with right hippocampal volumes, left temporal lobe volumes and right temporal lobe volumes. There was a trend to a negative correlation between 9am cortisol and left hippocampal volume (Spearman's $\rho = -0.19$, $N = 95$, $p = 0.065$). Post-dexamethasone cortisol levels were negatively and significantly correlated with right hippocampal volumes. There was also a significant negative correlation between 2.30pm cortisol levels and left temporal lobe volumes. None of the frontal lobe volumes correlated significantly in any direction with any of the cortisol measures, though the correlation between the left frontal lobe and 2.30pm cortisol approached statistical significance.

To date there are two published studies which have addressed these hypotheses (Lupien et al., 1998; Wolf et al., 2002), and both have had inadequate sample sizes. The present study provides support for the hypothesis that elevated glucocorticoids are negatively associated with hippocampal volumes and also suggest that glucocorticoid levels might also be associated with relatively smaller temporal lobes. A re-analysis of the above data using temporal lobe volumes with hippocampal volumes subtracted made no differences to the pattern of correlations.

Of interest is the fact that hippocampal volumes and not temporal lobe volumes appear to be more closely associated with post-dexamethasone cortisol levels. Although the sites in the brain which participate in feedback regulation of the HPA axis have not been fully delineated, with, for example, the medial frontal cortex

thought to play a role (Diorio et al., 1993), it is clear that the hippocampus plays an important role in feedback inhibition of the HPA axis. However, the studies with Cushing's syndrome show that elevated glucocorticoids probably cause widespread atrophy, in keeping with the widespread distribution of glucocorticoid receptors in the brain (Patel et al., 2000; Sanchez et al., 2000).

Because this is a cross-sectional study, it cannot be determined whether higher cortisol levels cause hippocampal atrophy and temporal lobe atrophy, or vice versa. It is possible that other variables contribute to ageing-related variations in brain volumes and cortisol levels. However, given the known causal effects of high levels of glucocorticoids on brain volumes in some animal studies it is plausible that the two variables are linked.

There were no significant correlations between cortisol levels and spectroscopy. These data suggest that variations in cortisol levels do not affect metabolite levels. Alternatively, in this healthy and young-elderly group, any effects of cortisol might be small. Follow-up studies will help to establish whether variations in cortisol levels are predictive of changes in metabolites.

There were few significant correlations between cortisol levels and cognitive function. 9am cortisol levels were significantly and negatively associated with the unadjusted scores of Raven's Standard Progressive Matrices and Logical Memory 24hr delayed, and when cognitive tests were adjusted for prior IQ, with the Digit-Symbol Substitution Test and Logical Memory 24hr delayed. Because there were several negative correlations between the cognitive tests and the cortisol measures, the cognitive factors were adjusted for prior IQ. To some extent this procedure reduces the amount of variance in the cognitive factors which is attributable to baseline general cognitive ability. This is of interest because the hypothesis is that elevated cortisol levels are associated with decrements in cognitive function with ageing, not with prior or baseline cognitive ability. The adjusted general cognitive factor and the adjusted fluid intelligence / visuospatial factor correlated significantly and negatively with 9am cortisol levels. Overall, these data are consistent with the hypothesis that there is a weak negative association between cortisol levels and

cognitive function in healthy men aged 65-70. Previous studies of comparable size have also shown relationships in the same direction and of similar effect size, though on different tests (Seeman et al., 1997; Greendale et al., 2000).

Some methodological limitations must be noted. This is a cross-sectional study and therefore the direction of causation, or any causation at all, cannot be inferred. The subjects were all male. The subjects are all volunteers and this is therefore not a population sample. Multiple analyses were carried out and this increases the risk of Type I statistical error. However, the hypotheses were made explicit from the outset, that is, these were planned analyses. Additionally, the relationships between cortisol levels and cognitive function were still statistically significant when principal components analysis was used to derive latent traits for cognitive function.

In summary, these data suggest that cortisol levels are negatively related to hippocampal and temporal lobe volumes in healthy men aged 65-70. The data are consistent with previous much smaller studies in humans, and with observational and interventional studies in animals. There were no significant relationships between metabolite levels and cortisol levels. Cognitive function showed weak and mostly statistically insignificant relationships in the predicted negative direction with 9am cortisol levels. Reducing the data with factor analysis and adjusting for prior IQ yielded a weak but statistically significant correlation between the first unrotated principal component, a variable accounting for half of the variance in the cognitive tests, and 9am cortisol levels. A similar significant correlation was found with the adjusted visuospatial / fluid factor.

This study adds to the small body of work examining the hypothesis that elevated glucocorticoid levels are negatively associated with the hippocampus and other brain structures, and also have a negative relationship with cognitive function. Future work will examine the impact of these variations in glucocorticoids on later changes in brain volumes and declines in cognitive function with ageing.

Chapter 7: Hyperglycaemia and cognitive function

7.1 Introduction

Type II diabetes mellitus is an established risk factor for Alzheimer's disease (Ott et al., 1999) and also milder cognitive decrements, specifically in verbal memory (Strachan et al., 1997). Impaired glucose tolerance in people without diabetes may also be associated with cognitive decline with age (Vanhanen et al., 1998). The metabolic syndrome, of which impaired glucose homeostasis is a key component, now has a prevalence of over 40% in people over 60 in the US (Ford et al., 2002). Glycosylated haemoglobin (HbA_{1c}) reflects the average blood glucose concentration over a 6-8 week period and is used in diabetic patients to monitor blood glucose control. However, in people without diabetes there is considerable variation in HbA_{1c}, which is predictive of other morbidity and mortality (Khaw et al., 2001). In this study the hypothesis that higher levels of HbA_{1c} are associated with relatively worse cognitive function in a group of healthy, unmedicated, non-diabetic elderly males is tested.

7.2 Methods

Recruitment of subjects was described in Chapter 2. The cognitive testing battery was also described in Chapter 2. A 9am morning specimen of blood was obtained for estimation of HbA_{1c} and plasma glucose, along with the other blood tests as described in Chapter 2.

7.3 Results

111 subjects were included in the study. The mean HbA_{1c} was 5.9% (SD 0.36; range 4.7-6.7) and the mean 9am plasma glucose was 5.1 mmol/l (SD 0.53; range 4.1 – 6.7). All variables were normally distributed and all reported correlations are Pearson's *r*. HbA_{1c} correlated significantly with 9am plasma glucose ($r=0.23$; $p=0.01$). HbA_{1c} had significant negative correlations with scores on the Rey Auditory-Verbal Learning Test ($r=-0.24$, $p=0.01$) and Logical Memory – 24 hr delayed ($r=-0.31$, $p=0.02$). Correlations with the remaining cognitive tests were not statistically significant, but were all in the same negative direction. Controlling for National Adult Reading Test scores, in case premorbid ability contributed substantially to the significant correlations, did not affect the pattern of the results. 9am plasma glucose did not correlate significantly with any of the cognitive tests. Correlations are shown in Table 7.1.

Table 7.1: Pearson correlations between cognitive tests, and glycosylated haemoglobin and fasting plasma glucose

	HbA1c	Glucose
National Adult Reading Test	-.13	-.04
Auditory-Verbal Learning Test	-.24 ^a	0.01
Logical Memory	-.16	-.04
Logical Memory – 24 hr delayed	-.31 ^b	-.07
Benton Visual Retention Test	-.03	-.06
Visual Reproduction	-.08	0.05
Verbal Fluency	-.13	-.09
Digit-Symbol Substitution Test	-.01	0.07
Raven's Standard Progressive Matrices	-.16	-.08

^ap=0.01, ^bp=0.018, 2-tailed.

Abbreviations: HbA1c: glycosylated haemoglobin (%); glucose: fasting plasma glucose (mmol/l)

7.4 Discussion

These findings suggest that, even within the notional normal range, relatively higher long-term blood glucose concentrations are negatively associated with two separate tests of verbal memory in healthy elderly men without diabetes. In this population HbA_{1c} levels explained about 5-9% of the variance in verbal memory. About half the variance in cognition in old age is explained by childhood cognitive ability (Deary et al., 2000b) and so it is important to identify the determinants of the large amount of change in cognition that takes place between childhood and old age. These results extend the established association between Type II diabetes mellitus and deficits in verbal memory, and add to the growing evidence that the components of the metabolic syndrome act as risk factors for vascular and other disease along continua, rather than beyond a particular level. Khaw and colleagues recently reported that, in a large population sample of non-diabetic men, HbA_{1c} levels are continuously associated with all cause, cardiovascular, and ischaemic heart disease mortality (Khaw et al., 2001). Similar data have recently been published for cholesterol (Stamler et al., 2000) and blood pressure (Vasan et al., 2001).

There are several possible explanations for the present association. In the shorter term this might reflect a pathogenic role of minor fluctuations in glucose homeostasis and/or its regulatory factors early in the process of ageing-related cognitive decline. This could involve attenuated forms of the metabolic and vascular changes of chronic hyperglycemia, or subtle glycosylation of CNS proteins, which associates with their dysfunction (Gispen and Biessels, 2000). These mechanisms may act over several decades, as neurons are post-mitotic. Another possible explanation is that the association is at least partially accounted for by a common causal antecedent such as low birthweight. Low birthweight is associated with a higher risk of insulin resistance later in life (Phillips et al., 1994) and has also been shown to be negatively associated with cognitive function in 11 year-olds (Shenkin et al., 2001) and in adults (Richards et al., 2001).

Certain methodological limitations should be noted. The sample size was modest, and the sample was non-random. The subjects were all male and relatively healthy and therefore the full spectrum of ill-health was not represented; however the experimental intention was to avoid possible disease or drug confounders. Multiple cognitive tests were performed and the significant correlations could represent Type I statistical error. However, it is persuasive that two different tests of verbal memory were significantly and negatively correlated with HbA1c, and it is this cognitive function that emerges from the literature as related to type 2 diabetes.

There is recent evidence that lifestyle measures can attenuate the tendency towards impaired glucose tolerance and type II diabetes (Knowler et al., 2002). Prospective studies will help to determine whether variations in glucose homeostasis are causally associated with variations in cognitive function, and if so, whether measures to correct impaired glucose homeostasis might impact on cognitive impairment in later life.

Chapter 8: General discussion

The studies in this thesis were aimed at understanding the links among three domains of variables: cognitive function, glucocorticoids and other metabolic variables, and brain structure and metabolite levels. The main hypothesis was that cortisol levels are negatively associated with both cognitive function and with hippocampal volumes in 111 healthy men aged 65 to 70, of whom 97 had usable neuroimaging data.

The study in Chapter 4 showed that, as in young adults, brain volumes are correlated with cognitive function, and that this relationship is best accounted for by a relationship between overall brain size and general cognitive ability rather than by relationships between specific brain regions and specific cognitive abilities (MacLulich et al., 2002). This is a potentially provocative finding, as the medical literature tends to ignore the concept of general cognitive ability or *g*, and also has tended not to examine the possibility that such general relationships exist.

Brain metabolite levels, as measured by magnetic resonance spectroscopy, also showed relationships with some cognitive ability tests (Ferguson et al., 2002). Specifically, choline / creatine and NAA / creatine ratios were positively correlated with performance on verbal and visuospatial tests and adjusted creatine levels also correlated negatively with performance on these tests. Because these metabolite levels are not absolute, it cannot be determined which is or are responsible for these correlations. However, the pattern of correlations and also the correlations with adjusted creatine levels suggest that perhaps creatine levels are responsible for some of the variance in cognitive ability. This is an interesting finding, as most investigators make the assumption that of the metabolite levels measured, creatine shows the least change in pathological conditions and in ageing and thus is used as the denominator in metabolite ratios (Catani et al., 2001). However, a small number of studies in the literature do suggest the possibility that creatine levels may change with ageing (Pettegrew et al., 1990; Pettegrew et al., 1995), and studies with spectroscopic techniques allowing for absolute quantification of metabolite levels will help to address this issue.

There are few studies which have examined relationships between cognitive function and glucocorticoid variables in humans. Research combining measurements of cognitive function, glucocorticoids and neuroimaging data is even more scarce, with one small published study in healthy elderly individuals (Lupien et al., 1998), and one in patients with Cushing's syndrome (Starkman et al., 1992). In the present study, cortisol levels showed some small, statistically significant negative correlations with hippocampal and temporal lobe volumes. There was also a smaller number of small, statistically significant negative correlations with cognitive function, though these correlations were only with 9am cortisol and only present when premorbid or prior IQ was controlled for. However, the general cognitive factor and the rotated visuospatial / fluid factor, adjusted for prior IQ, also showed small but significant negative correlations with 9am cortisol, suggesting that this may be a true finding. These data are consistent with animal studies, which have tended to show that there is an association between higher glucocorticoid levels and cognitive decrements with ageing, though this is not a large effect, and the mechanisms responsible for this association remain unclear (Seckl and Olsson, 1995; Yau et al., 2002).

Diabetes mellitus is a risk factor for Alzheimer's disease (Ott et al., 1999) and for milder cognitive decline (Strachan et al., 1997). Relative hyperglycaemia in the non-diabetic range has been linked with cardiovascular mortality and morbidity (Khaw et al., 2001). In this thesis glycosylated haemoglobin and fasting glucose were correlated against cognitive function. Glycosylated haemoglobin correlated significantly and negatively with performance on two different tests of declarative memory; there were no significant correlations with fasting glucose. In the context of the small effect size of hyperglycaemia on cognitive function (Strachan et al., 1997) this is a small study, but these results are consistent with the hypothesis that relative hyperglycaemia is associated with ageing-related cognitive decrements, though causation cannot be inferred. At this stage, this study can be seen as a prompt for larger-scale, longitudinal studies examining this association. Hyperglycaemia is of particular interest as a risk factor because there is already evidence that lifestyle

changes and treatment with widely-available drugs are effective in improving glucose tolerance (Knowler et al., 2002).

There are certain methodological limitations applying to all of the studies in this thesis which must be noted. All of the investigations are cross-sectional. This means that it cannot be known whether any of the significant relationships are of recent onset (that is, are related to ageing) or whether they are longstanding. The author and collaborators plan to repeat the measurements made in this study in the near future and in subsequent waves. The participants were not selected randomly and were all healthy and male, therefore are not representative of the population. Although this is a large study in comparison to most studies involving neuroimaging or glucocorticoid measurements in humans, the correlations observed are relatively small. Associations between neuroimaging and glucocorticoid variables and cognitive function are not large and therefore this study is perhaps at the lower limit of acceptable size.

Future Work

Cognitive ageing research has to date largely consisted of two broad domains: measuring and characterising the variations in cognitive function with ageing, and investigating the biological substrates of these variations. A third domain, which has yet to develop in any substantial sense in human studies, is the investigation of measures which prevent ageing-related decrements in cognitive function.

The measurement and characterisation of cognitive function in healthy adults at a particular point in time, that is, baseline cognitive function, is well-established. There are many reliable and valid tests available, and the underlying statistical structure of individual differences in cognitive function is well understood (Carroll, 1993; Deary, 2001a). However, the characterisation of changes in cognitive function with ageing is less clear. Certainly, there are mean declines in performance on many tests, particularly those involving cognitive speed and the manipulation and storage of new information. This phenomenon has been conceptualised in the work of Salthouse, in whose theory a single latent trait termed 'processing speed' accounts for much of the age-related variance in cognitive ability (Salthouse, 1985; Salthouse et al., 1996), and Cattell (Cattell, 1963), who introduced the constructs of fluid intelligence and crystallised intelligence.

However, much research in cognitive ageing, particularly that published in medically-orientated journals, operates without reference to the empirical and conceptual bases of psychometric psychology. This other main strand of cognitive ageing research draws on the neuropsychological and cognitive psychology traditions; essentially, this strand examines changes in various cognitive domains without considering latent traits. It has tended to be focused on either changes in cognitive functions thought to be dependent on the frontal lobes (West, 1996), or on declarative memory (Jelicic et al., 1996). Many of the studies in this approach are concerned with the prediction of dementia and/or examining relationships between specific cognitive tests and specific brain regions (deLeon et al., 1997; Golomb et al., 1993).

This theoretical divide is curious, not least because there is little debate or even awareness of its existence; each body of research seems to operate almost independently of each other. An important consequence of this is that there are very few studies using the latent trait approach in attempting to discover patterns of cognitive decline predictive of dementia and to the author's knowledge there are no published studies of neuroimaging and cognitive function in older populations which have employed principal components analysis or factor analysis, other than the study presented in Chapter 4 (MacLulich et al., 2002). Progress in cognitive ageing research would be aided by at least the beginnings of a debate in the medical literature about what might constitute the basics of a common conceptual and methodological framework for cognitive ability at baseline and with ageing. There are some recent documents which have started this process (Deary, 2001b; National Research Council, 2000).

There are still several unresolved issues in terms of the measurement and characterisation of cognitive function with ageing. Although many cognitive tests show a mean decline and an increase in variance with ageing (Christensen et al., 1999), it is not clear whether there are identifiable clusters of impairment affecting one or more domains or whether there is a more-or-less continuous spectrum of change. It can be hypothesised that there is a universal but varying amount of relatively steady decline in cognitive functions which to some extent can be placed under the general heading of 'fluid intelligence'. This is the typical finding in psychometrically-orientated studies, which mainly study healthy people. There are also one or more syndromes of often much more rapid and severe declines in cognitive function, perhaps particularly in declarative memory, which may represent the early part of a trajectory towards dementia. There is evidence to support this hypothesis from prospective studies of cognitive function (Collie et al., 2001; Elias et al., 2000), neuroimaging studies, which have shown that subjects who later develop Alzheimer's disease dementia show much more rapid loss of brain volumes than controls (Jobst et al., 1994; Fox et al., 1996), and pathological studies, which show that there are large differences in pathological changes in patients with even early Alzheimer's disease versus controls (Morris and Price, 2001). It is not known if there

are intermediate levels of decline which also have functional significance; in the medical literature there have been attempts to characterise possible intermediate states using various terms and definitions, including age-associated memory impairment, mild cognitive decline and so on, though the somewhat arbitrary nature of these definitions has been criticised (Deary, 1995; Ritchie and Touchon, 2000). Future studies in this field are only likely to be of use if they are longitudinal, large-scale, population-based and use sensitive, reliable and valid cognitive tests. Furthermore, an open empirical approach is required when trying to understand such changes: it is conceivable that traditional ways of classifying individual differences in cognitive function, for example, into domains such as executive function, declarative memory, and so on, will turn out to be inappropriate.

Integration of detailed cognitive testing with detailed neuroimaging techniques is also likely to yield much information about the patterns of brain ageing. For example, the use of registration and digital subtraction of serially-collected brain images, as developed by Fox and co-workers (Fox et al., 1996), in conjunction with detailed cognitive testing, could help to answer the important question of whether there is a syndrome of relatively specific decline in memory and atrophy of medial temporal lobe structure. There are dozens of small studies which suggest this but, to this author's knowledge, none which has the minimum required characteristics of being population-based, longitudinal (with a starting age of under 60), having detailed cognitive testing, adequate statistical power and using appropriate statistical methods. Until such a study or studies are undertaken, attempts to classify variations in cognitive ageing in relation to changes in the brain will remain speculative and of minimal clinical benefit.

A large number of putative influences on cognitive ageing have been studied, often in the context of the prediction of the dementias. For example, the $\epsilon 4$ allele of the apolipoprotein E gene is associated with a higher risk of Alzheimer's disease (Corder et al., 1993) but also milder cognitive decline (Deary et al., 2002; MacLulich et al., 1998). Other potentially important associations include common illnesses such as hypertension (Waldstein, 1995), hyperlipidaemia (Fillit et al., 2002) and diabetes

mellitus (Elias et al., 1997; Strachan et al., 1997; Ott et al., 1999). There is likely to be another category of processes which are less well understood, for example, changes in the permeability of the blood-brain barrier with ageing (Blatt et al., 1997; Hanyu et al., 2002). Initial efforts to understand such candidate processes will usually involve small studies, but it is still important that standard sensitive and reliable cognitive tests are used.

The findings from this thesis are relevant to important areas of cognitive ageing research, including the use of statistical techniques which allow for the analysis of latent traits, and the relationships of variations in cognitive abilities to structural neuroimaging and magnetic resonance spectroscopy variables. In terms of mechanisms, the findings are consistent with an association of elevated glucocorticoids with decrements in cognitive function. Further research, including further investigations of the cohort studied in this thesis, will help to establish whether glucocorticoids are significantly associated with variations in cognitive ageing and underlying changes in the brain.

Appendices

Appendix 1: Neuroimaging protocols

Structural magnetic resonance imaging

Analysis of volumes was performed by Dr. Karen J. Ferguson.

Magnetic Resonance Imaging: Brain imaging was performed in an Elscint Prestige MR scanner operating at 1.9T. Structural image acquisition followed a 3-view localiser and consisted of a coronal T₁-weighted three-dimensional gradient echo sequence covering the entire brain and skull (TE = 9.254, TR = 28.5, tip angle = 25°, slice thickness = 1.5mm (no interslice gap), 18cm field of view, matrix = 180 x 180).

Image analysis:

Image analysis was carried out on Sun workstations using AnalyzeTM software (Mayo Clinic, Rochester, MN). An intensity threshold separating the brain from the meninges was imposed for semi-automated analysis.

Hippocampal formation: this was defined as subiculum, hippocampus proper and dentate gyrus with the alveus and fimbria. The hippocampus was measured bilaterally using manual tracing from the first slice in which it appeared until the full extent of the crus fornicis appeared. In the posterior head of the hippocampus, an arbitrary judgement of the boundary between the hippocampus and the amygdala was made from the position of the temporal horn and the alveus or by comparing a slice with subsequent slices in which the division was more obvious. A line was drawn at the angle between the superior and medial surfaces of the parahippocampal gyrus to separate the subiculum from the parahippocampal gyrus. Intra-rater error was 4±

0.03% for left and $4 \pm 0.04\%$ for right mean hippocampal volumes; this was within limits identified in previous studies.

Prefrontal lobe: measured from the slice in which the frontal pole could be distinguished from the meninges. Measurements were made using automated methods with manual tracing to separate the lobes through the inter-hemispheric fissure. The last slice was that before the appearance of the genu of the corpus callosum.

Temporal lobe: the anterior part of the temporal lobe was measured semi-automatically. Subsequently, a line was drawn manually along the lateral fissure to the Sylvian point and then diagonally to the superior-most point of the amygdalo-hippocampal complex and along the superior boundary of this structure. The last slice of the temporal lobe was the same as for the hippocampus.

Intracranial area (ICA): this was measured in the midline sagittal slice of the sagittal localiser by manually tracing round the inner table of the cranial vault, along the superior surface of the floor of the frontal fossa and across the pituitary fossa to the dorsum sella. Tracing continued down the posterior surface of the clivus and was completed by a line joining the anterior and posterior rims of the foramen magnum.

Magnetic Resonance Spectroscopy

Analysis of spectra was performed by Dr. Karen J. Ferguson, Professor Joanna M. Wardlaw and Dr. Ian Marshall.

All MR scanning was performed on an Elscint Prestige scanner operating at 1.9T (Elscint, now GE Medical, Israel). Spectroscopy was carried out following structural imaging. Placement of the volume of interest (VOI) for spectroscopy was carried out using the structural images acquired for volumetric analysis in the coronal plane. The VOI was a voxel (15mm x 15mm x 15mm) placed over the parietal lobe and included mainly white matter with some grey, but avoided any contamination from

non-cerebral tissue. Following shimming on the VOI, water-suppressed, PRESS-localized ^1H signals (TR/TE = 1500/145 ms) were acquired with 200 repetitions ("number of excitations"; NEX). Phase reference signals (8 NEX) were acquired from the same VOIs without water suppression. The time domain signals sampled the second half of the PRESS echo and consisted of 1024 complex data points sampled at 1 ms intervals.

Spectral quantification

Spectroscopic data were transferred to a network of Sun workstations (Sun Microsystems, Mountain View, CA, USA). The water reference signals were used for time domain correction of eddy current effects (Ordidge and Cresshull, 1986), and the residual water signal removed by Hankel-Lanczos Singular Value Decomposition (HLSVD) identification of water components (Van den Boogart et al., 1994). Signals were then transformed to the frequency domain. Spectral peaks areas corresponding to the metabolites choline, creatine and N-acetylaspartate (NAA) were quantified in the frequency domain, fitting for frequencies, areas and line widths with a Gaussian lineshape using in-house software (Marshall et al., 2000). No attempt was made to relate fitted peak areas with absolute metabolic concentrations.

Appendix 2: Auditory-Verbal Learning Test word lists

List A

drum
curtain
bell
coffee
school
parent
moon
garden
hat
farmer
nose
turkey
colour
house
river

List B

desk
ranger
bird
shoe
stove
mountain
glasses
towel
cloud
boat
lamb
gun
pencil
church
fish

Appendix 3: Histograms of distributions of cognitive tests

Figure A3.1: Histograms of raw scores from the cognitive tests

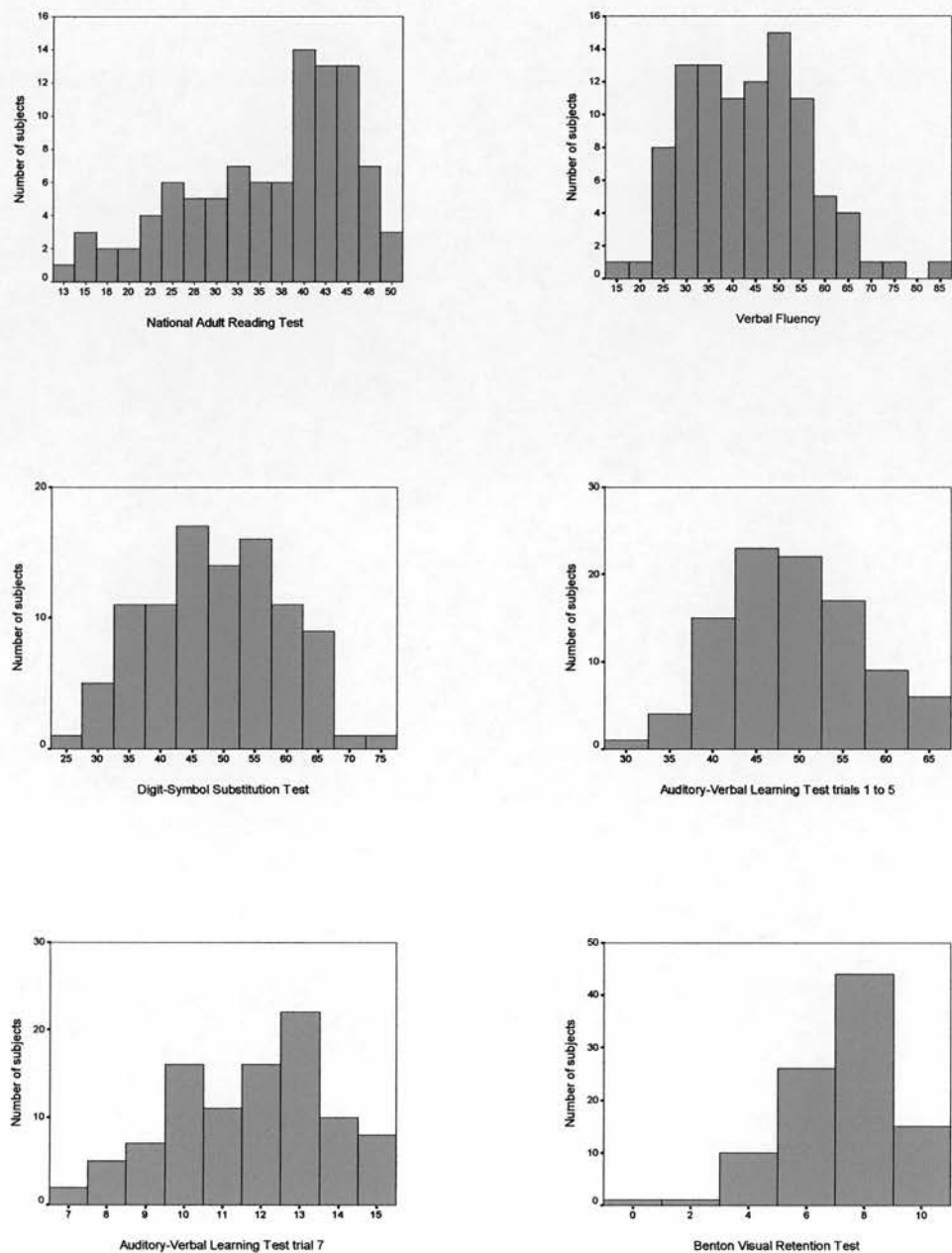
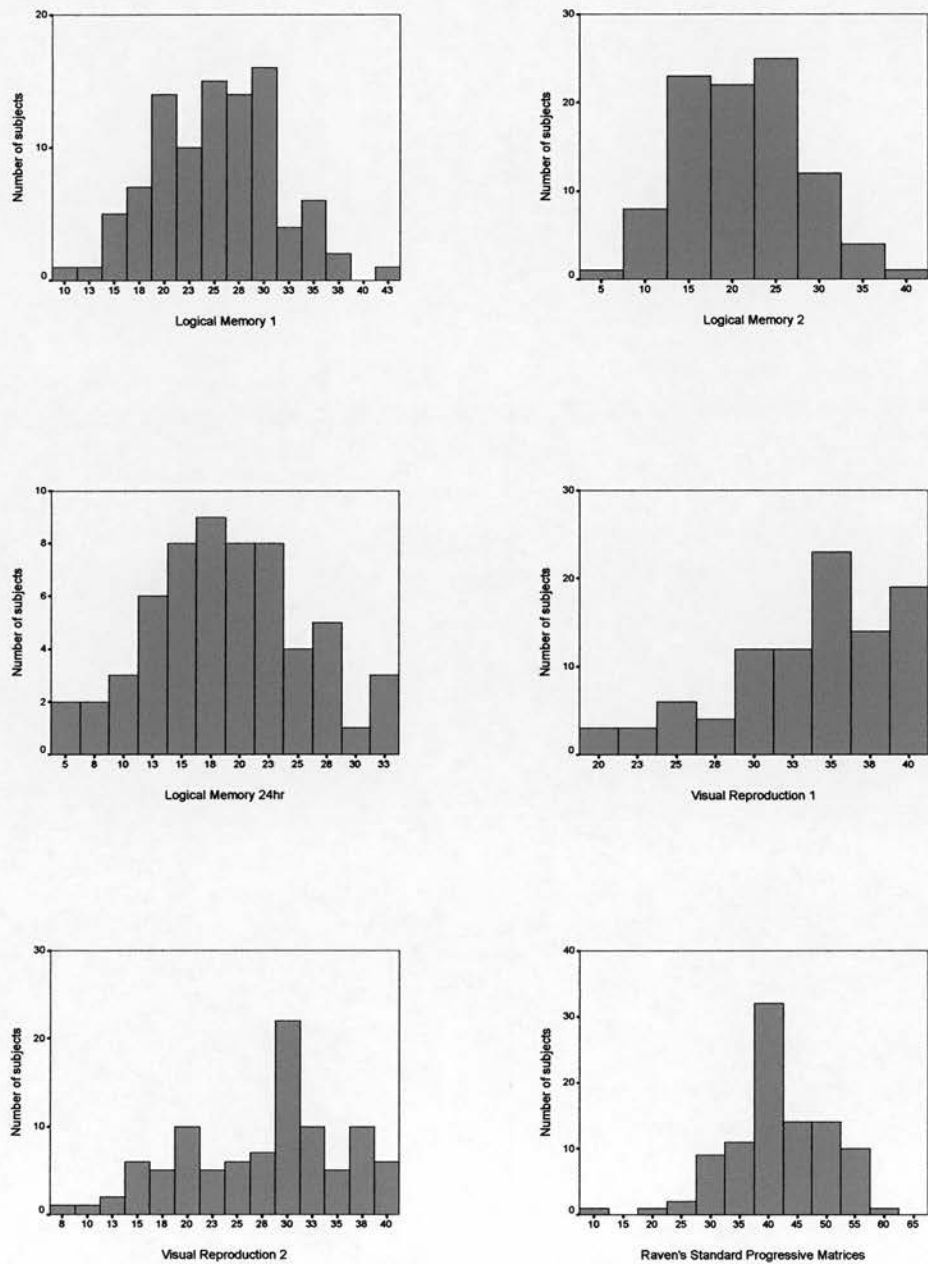


Figure A3.1 (cont). Histograms of raw scores from the cognitive tests



Appendix 4: Spearman correlations among cortisol levels

	2.30pm cortisol	Post-dex cortisol
9am cortisol	.26 (p=0.017, N=86)	.22 (p=0.039, N=88)
2.30pm cortisol		.06 (p=0.061, N=84)

Appendix 5: Glossary of technical terms

Crystallised intelligence: intelligence involving the ability to recall and use previously learned information.

Direct Oblimin rotation: in factor analysis, a method of obtaining correlated (oblique) factors in order to approximate simple structure.

Eigenvalue: Column sum of squared loadings for a factor; also referred to as the latent root. It conceptually represents that amount of variance accounted for by a factor.

Fluid intelligence: intelligence involving processing of novel information.

g: general intelligence.

Latent variable: a theoretical variable hypothesized to influence a number of observed variables. This term is synonymous with Latent Trait.

Principal components analysis: a data reduction method used to identify patterns in a data matrix. This method yields a smaller set of new orthogonal (non-correlated) latent variables.

Shimming: a procedure carried out to optimise the homogeneity of the magnetic field around the subject during magnetic resonance spectroscopy.

Volume of Interest (VOI): In magnetic resonance spectroscopy, the volume selected for measurement.

Voxel: volume element; the element of 3-D space corresponding to a pixel, for a given slice thickness.

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